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Modelling of vitamin A binding to tRNA

C.N. N'soukpoé-Kossi, P. Bourassa, J.S. Mandeville, H.A. Tajmir-Riahi*

Department of Chemistry-Physics, Université du Québec à Trois-Rivières, C. P. 500, Trois-Rivières, Québec, Canada G9A 5H7

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ABSTRACT

The binding sites of retinol and retinoic acid with tRNA are located in aqueous solution at physiological conditions using constant tRNA concentration and various retinoid contents. FTIR, CD, fluorescence spectroscopic methods and molecular modelling were used to determine retinoid binding sites, the binding constant and the effects of retinol and retinoic acid complexation on tRNA conformation and aggregation. Structural analysis showed that retinol and retinoic acid bind tRNA *via* G–C and A–U base pairs with overall binding constants of $K_{ret-tRNA} = 2.0\ (\pm 0.40) \times 10^4\ M^{-1}$ and $K_{retac-tRNA} = 6.0\ (\pm 1) \times 10^4\ M^{-1}$. The number of binding sites occupied by retinoids on tRNA were 1.4 for retinol–tRNA and 1.7 for retinoic acid–tRNA complexes. Hydrophobic interactions were also observed at high retinol and retinoic acid contents. Molecular modelling showed the participation of several nucleobases in retinoid–tRNA complexation with free binding energy of −4.36 for retinol–tRNA and −4.53 kcal/mol for retinoic acid–tRNA adducts.

1. Introduction

13-*cis* Retinoic acid is rapidly absorbed into cells and exerts its anti-proliferative effect on human sebocytes by specific isomerization to high levels of all-*trans* retinoic acid (Scheme 1) and binding the retinoic acid receptors [1,2]. DNA adducts have been widely used to identify the health hazards and to evaluate the dose–response relationship in human exposed to carcinogens and mutagenic compounds [3,4]. Dietary constituents of fresh fruits and vegetables might play a relevant role in DNA adduct formation by inhibiting enzymatic activities [5,6]. Antioxidant micronutrients have been shown to prevent DNA damage caused by polycyclic aromatic hydrocarbon and other carcinogens and to alter expression of metabolic enzymes [6,7]. Many studies have addressed the role of antioxidant vitamins A, B and C in protection against cancer and cardiovascular diseases [8]. It has been suggested that the antioxidant activity of retinol, retinoic acid (Scheme 1) and beta-carotene includes scavenging free radicals and preventing DNA damage [9–11]. The effect of vitamin A on cleavage of plasmid DNA has been recently reported [12]. Beta-carotene radical was found to intercalate DNA duplex [13]. Vitamin A components, retinol and retinoic acid, are fat-soluble micronutrients and critical for many biological processes, including vision, reproduction, growth, and regulation of cell proliferation and differentiation [14,15]. Since vitamin A components play a role in DNA adduct formation thus, interaction of tRNA with retinol and retinoic acid is of a major biological importance.

We now report the structural analysis of tRNA complexes with retinol and retinoic acid by FTIR, CD, fluorescence spectroscopic methods and molecular modelling. Structural information regarding the retinoid binding sites, binding constant and the effects of retinoid on tRNA stability and secondary structure are provided. This is the first spectroscopic and structural analysis of tRNA interaction with retinol and retinoic acid at molecular level, which can contribute to elucidating the nature of this biologically important complexation.

2. Experimental

2.1. Materials

All-*trans* retinol and all-*trans* retinoic acid were purchased from Sigma Chemical Company. 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 [16].

Abbreviations: ret, retinol; retac, retinoic acid; FTIR, Fourier transform infrared; CD, circular dichroism.

* Corresponding author. Tel.: +1 819 376 5011x3310; fax: +1 819 376 5084.
E-mail addresses: tajmirri@uqtr.ca, heidar-ali.tajmir-riahi@uqtr.ca (H.A. Tajmir-Riahi).

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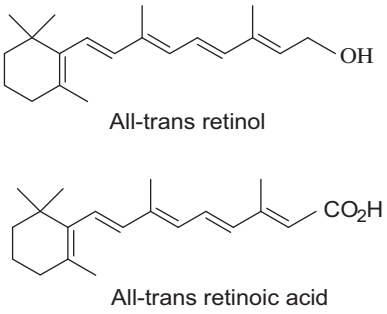

Scheme 1. Chemical structures of retinol and retinoic acid.

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) [17]. 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. The solutions of retinol and retinoic acid (0.25 μM to 2 mM) were prepared in 25% ethanol/water. The retinoid solution was added drop-wise to tRNA solution with constant stirring to ensure the formation of homogeneous solution. It is worth noting that ethanol content of 25% in the mixtures used to dissolve retinol and retinoic acid does not affect the conformation of tRNA. Ethanol induces polynucleotides conformational changes (B to A-form) when the alcohol concentration exceeded 50% [18].

2.3. FTIR spectroscopic measurements

Infrared spectra were recorded on a BOMEM DA3-0.02 Fourier transform infrared spectrometer, equipped with a nitrogen cooled HgCdTe detector and a KBr beam splitter. Solution spectra were recorded in solution on AgBr windows with resolution of 2 cm^{-1} and 100 scans. The retinoid concentrations used in infrared were 0.125, 0.25, 0.5 and 1 mM with final tRNA content of 12.5 mM (P). The water subtraction was carried out with 0.1 M NaCl solution used as a reference at pH 7.3 [19]. A good water subtraction was achieved when a flat baseline was obtained around 2200 cm^{-1} where the water combination mode is located. This method is a rough estimate, but removes the water content in a

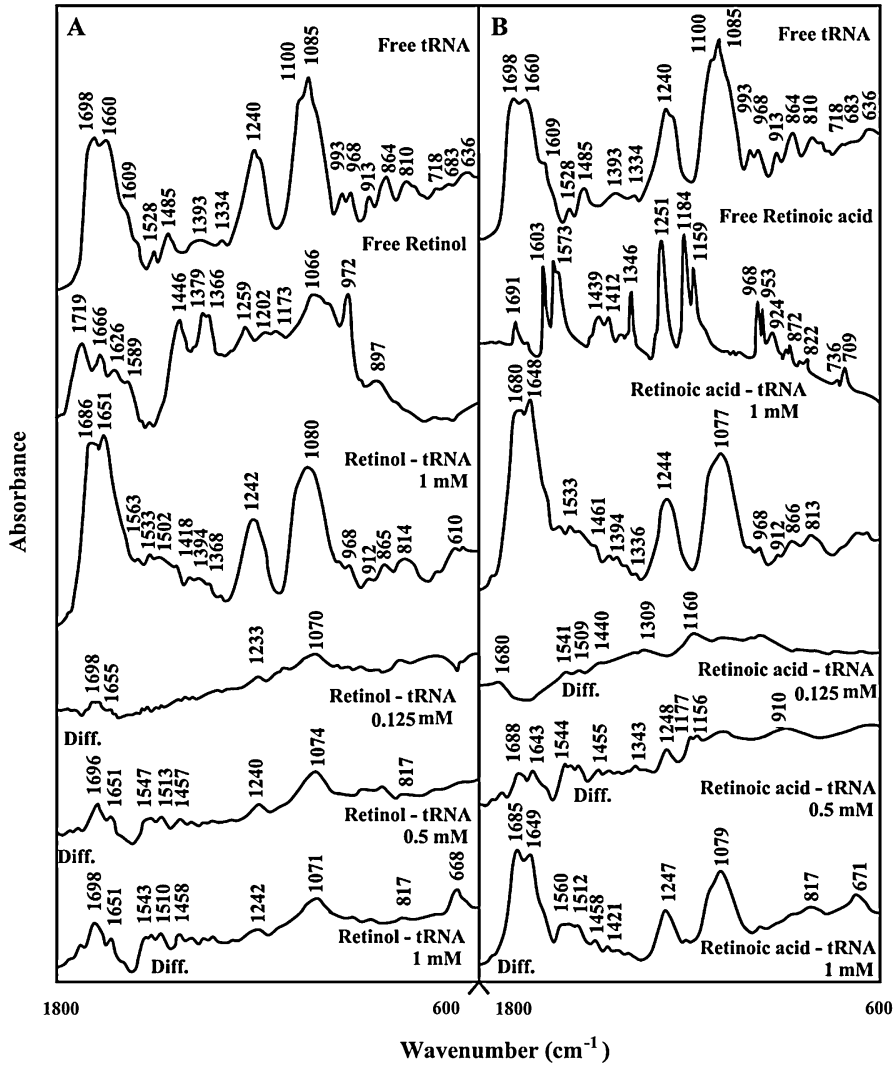

Fig. 1. FTIR spectra and difference spectra [(tRNA solution + retinoid solution) – (tRNA solution)] in the region of 1800–600 cm^{-1} for the free tRNA and its retinol (A) and retinoic acid (B) complexes in aqueous solution at pH 7.4 with various retinoid concentrations and constant tRNA concentration (12.5 mM).