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Interaction of zanamivir with DNA and RNA: Models for drug–DNA and drug–RNA bindings

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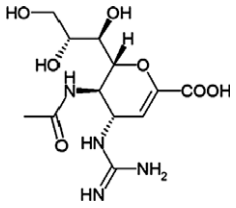
Abstract

Zanamivir (ZAN) is the first of a new generation of influenza virus-specific drugs known as neuraminidase inhibitors, which acts by interfering with life cycles of influenza viruses A and B. It prevents the virus spreading infection to other cells by blocking the neuraminidase enzyme present on the surface of the virus. The aim of this study was to examine the stability and structural features of calf thymus DNA and yeast RNA complexes with zanamivir in aqueous solution, using constant DNA or RNA concentration (12.5 mM) and various zanamivir/polynucleotide (*P*) ratios of 1/20, 1/10, 1/4, and 1/2. FTIR and UV–visible spectroscopy are used to determine the drug external binding modes, the binding constant and the stability of zanamivir–DNA and RNA complexes in aqueous solution. Structural analysis showed major interaction of zanamivir with G-C (major groove) and A-T (minor groove) base pairs and minor perturbations of the backbone PO_2 group with overall binding constants of $K_{\text{zanamivir–DNA}} = 1.30 \times 10^4\ M^{-1}$ and $K_{\text{zanamivir–RNA}} = 1.38 \times 10^4\ M^{-1}$. The drug interaction induces a partial B to A-DNA transition, while RNA remains in A-conformation.

Keywords: DNA; RNA; Zanamivir; Binding constant; Binding mode; Conformation; Stability; Secondary structure; FTIR; UV–visible spectroscopy

1. Introduction

Zanamivir (relenza) (5-acetamido-4-guanidino-6-(1, 2, 3-trihydroxypropyl)-5,6-dihydro-4*H*-pyran-2-carboxylic acid) also known as GG167 (structure 1) was the first potent inhibitor of influenza virus sialidase (also known as neuroaminidase NA) to be discovered [1,2].



It is antiviral agent and has been shown to inhibit viral replication in vitro [1] and *in vivo* (mouse and ferret models) [3] and in experimental infections in human volunteers. These effects have been reviewed by Calfee and Hayden [4–6]. Relenza inhibits all influenza type A and B. Influenza virus is highly infectious and can cause worldwide pandemics with significant morbidity and mortality [1,5,7,8]. Relenza works by interfering with the life cycles of influenza viruses A and B. It prevents virus spreading infection to other cells by blocking the neuroaminidase enzyme present on the surface of the virus and binds only to highly conserved amino acids within the active site of the neuroaminidase enzyme. Relenza adopts a position within the active site of the enzyme and copies the geometry of the sialoside hydrolysis transition state. It can achieve very good binding through appropriate presentation of its four pendent substituents and contains a hydrogen bonding glycerol side

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chain. The guanidine group in relenza is believed to form salt bridges with Glu 119 in the neuroaminidase active site and add a strong charge interaction with Glu227. Two hydroxyl groups of the 6-glycerol side chain are hydrogen bonded to Glu276 and the 4-hydroxyl is oriented towards Glu119. The NH group of the 5-*N*-acetyl side chain interacts with a bound water molecule on the floor of the active site. The carbonyl oxygen of the same side chain is hydrogen bonded to Arg152 and the methyl group enters a hydrophobic pocket lined by llc222 and Trp178. The glycosidic oxygen projects into bulk solvent [9,10]. Zanamivir does not inhibit other viral, bacterial or human neuroaminidase enzyme to any significant extent. It is an effective, potent inhibitor of influenza virus replication in vitro [11]. However, there has been no report on the interaction of zanamivir with DNA and RNA in aqueous solution. Therefore, it was of our interest to examine the binding sites of zanamivir to DNA and RNA bases and the backbone phosphate group.

We now report the results of FTIR and UV–visible spectroscopic analysis of DNA and RNA interactions with zanamivir in aqueous solution at physiological conditions using constant DNA and RNA concentrations (12.5 mM) and various drug–DNA and -RNA phosphate ratios of 1/20 to 1. The drug binding site, the binding constant and the effects of drug complexation on the stability and conformation of DNA and RNA are discussed here.

2. Methods and materials

2.1. Materials

Highly polymerized type I calf thymus DNA sodium salt (7% Na content) and Baker's yeast RNA sodium salt were purchased from Sigma Chemical Co. and used without further purification. To check the protein content of DNA and RNA solutions, the absorbance bands at 260 and 280 nm were used. The A_{260}/A_{280} ratio was 1.80 for DNA and 2.1 for RNA, showing that the DNA and RNA samples were sufficiently free from protein [12]. Zanamivir was obtained from Glaxowelcom. Other chemicals were of reagent grade and used without further purification.

2.2. Preparation of stock solutions

Sodium–DNA or sodium–RNA was dissolved to 1% w/v (25 mmol polynucleotide (phosphate)/L) in an aqueous solution containing 0.1 M NaCl and 1 mmol sodium cocodylate/L (pH 7.30) at 5 °C for 24 h with occasional stirring to ensure the formation of a homogeneous solution. The appropriate amount of zanamivir (0.3–25 mmol/L) was dissolved in water. The drug solution was then added dropwise to DNA or RNA solution to attain desired zanamivir/DNA(P) and zanamivir/RNA(P) molar ratios of 1/20, 1/10, 1/5, 1/2 and 1/1 at a final DNA or RNA concentration of 0.5% w/v (12.5 mmol polynucleotide (phosphate)/L) for infrared measurements. The final concentrations of the calf thymus DNA and RNA solutions were determined spectrophotometrically at 260 nm using molar extinction coefficient $\varepsilon_{260} = 6600\ \mathrm{cm^{-1}M^{-1}}$ (expressed as molarity of phosphate group [13,14]).

2.3. Absorption spectroscopy

The absorption spectra were recorded on a LKB model 4054 UV–vis spectrometer, using various pigment concentrations (5 μM to 0.05 mM) and DNA or RNA concentration of 0.1 mM.

2.4. FTIR spectroscopic measurements

Infrared spectra were recorded on a Jasco FTIR spectrometer equipped with a liquid-nitrogen-cooled HgCdTe (MCT) detector and a KBr beam splitter. The spectra of the zanamivir/polynucleotide solutions were acquired using a cell assembled with AgBr windows. Spectra were collected after 2 h incubation of zanamivir with the polynucleotide solution and were measured in triplicate (three individual samples of the same polynucleotide and zanamivir concentrations). For each spectrum, 100–500 scans were collected at a resolution of 2 to 4 $\mathrm{cm^{-1}}$. The water subtraction was carried out using an NaCl (0.1 mol/L) solution at pH 6.5–7.5 as a reference. A good water subtraction was considered to be achieved when there was a flat baseline around 2200 $\mathrm{cm^{-1}}$, where the water combination mode is located. This method yields a rough estimate of the subtraction scaling factor, but removes the spectral features of water in a satisfactory way [15]. The difference spectra [(polynucleotide solution + zanamivir solution) − (polynucleotide solution)] were obtained using a sharp DNA band at 968 $\mathrm{cm^{-1}}$ and an RNA band at 864 $\mathrm{cm^{-1}}$ as internal references. These bands, which are due to sugar C–C and C–O stretching vibrations, exhibit no spectral changes (shifting or intensity variations) upon zanamivir–polynucleotide complexation, and they were cancelled out upon spectral subtraction [16]. The intensity ratios of bands due to several DNA or RNA in-plane vibrations related to A-T, A-U, and G-C base pairs and the PO_2 stretching vibrations were measured with respect to the reference band at 968 (DNA) or 864 $\mathrm{cm^{-1}}$ (RNA) as a function of zanamivir concentration with an error of ±3%. Similar intensity variations have been used to determine the drug binding to DNA and RNA bases and backbone phosphate groups [17–19].

3. Results and discussion

3.1. FTIR spectra of zanamivir–DNA complexes

Evidence related to the zanamivir–DNA complexation comes from the infrared spectroscopic results shown in Figs. 1 and 2A. At low zanamivir concentration ($r = 1/20$), minor drug–DNA interaction occurs. Evidence for this comes from the spectral changes (intensity or shifting) of

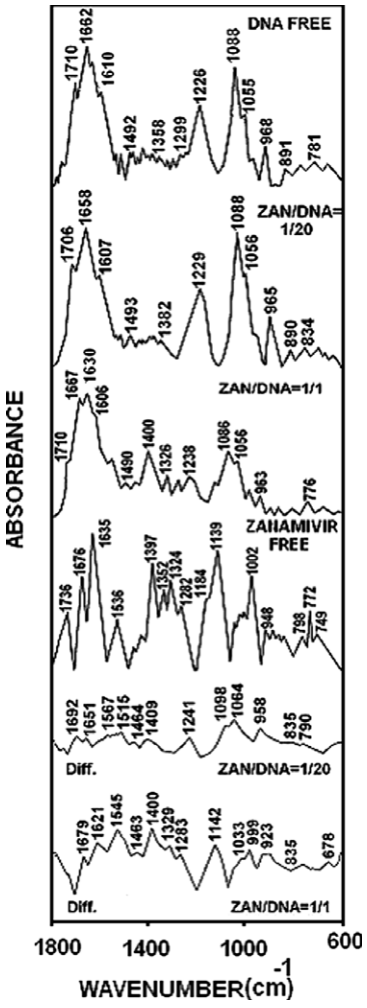

Fig. 1. FTIR spectra in the region of 1800–600 cm^{-1} for free calf thymus DNA, zanamivir, and zanamivir adducts in aqueous solution at pH 6.5–7.5 (top four spectra) and difference spectra obtained at various zanamivir–DNA (phosphate) molar ratios (bottom two spectra).

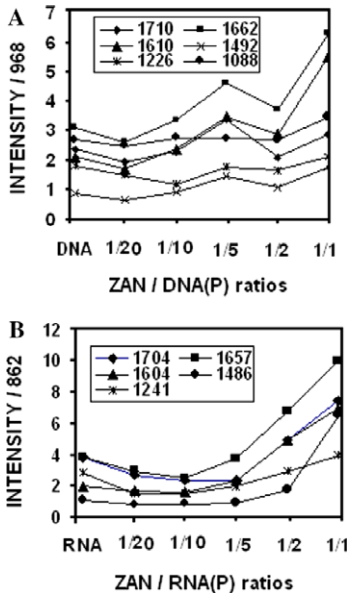

Fig. 2. Intensity ratio variations for several DNA and RNA in-plane vibrations as a function of zanamivir concentration. (A) Intensity ratios for the DNA bands at 1710 (G, T), 1662 (T, G, A, C), 1610 (A), 1492 (C,G), 1226 (PO_2 asymmetric stretch), and 1088 cm^{-1} (PO_2 symmetric stretch) referenced to the DNA band at 968 cm^{-1}; (B) intensity ratios for the RNA bands at 1704 cm^{-1} (G, U), 1657 cm^{-1} (U), 1604 cm^{-1} (A), 1486 cm^{-1} (G, C), and 1241 cm^{-1} (PO_2 stretch) referenced to RNA band at 864 cm^{-1}.

several polynucleotides in-plane vibrations related to the G-C, A-T bases and the backbone phosphate modes at 1710 (mainly G), 1662 (mainly T), 1610 (A), 1492 (mainly C), and 1226 (asymmetric PO_2 vibration) and 1088 cm^{-1} (PO_2 symmetric vibration) [15,16,20–24] in the presence of zanamivir (Figs. 1 and 2A).

At $r = 1/20$, shifting for the bands at 1710 (guanine) to 1706, 1662 (thymine) to 1658, 1610 (adenine) to 1607, 1492 (cytosine) to 1493, and 1226 (asymmetric stretching phosphate) to 1229 cm^{-1} was observed (Fig. 1). The observed shifting was accompanied by a decrease in intensities of guanine band (7%), thymine band (5%), adenine band (8%), cytosine band (14%), PO_2 (asym) (7%), and PO_2 (sym) (5%) (Fig. 2A). A minor loss of intensity of the DNA in plane vibrations can be attributed to a partial helix stabilization as a result of drug–DNA complexation. Similar spectral changes were observed in the spectrum of quercetin–DNA [25] in which minor loss of intensity of the DNA in plane vibrations attributed to a partial helix stabilization, while the increase in intensity of DNA vibrations at high drug content can be attributed to some degree of helix destabilization [26,27].

As zanamivir concentration increased ($r = 1/10$), shiftings for the bands at 1710 (guanine) to 1703, 1662 (thymine) to 1656, 1610 (adenine) to 1606, 1492 (cytosine) to 1494, 1226 (PO_2 asymmetric) to 1229, and 1088 (PO_2 symmetric) to 1087 cm^{-1} were observed in the spectra of zanamivir–DNA complexes. Some increases in the intensities of adenine, guanine, cytosine and thymine bands were observed. The spectral changes can be related to zanamivir interaction with G-C and A-T base pairs. The phosphate bands at 1226 (asym PO_2), 1088 cm^{-1} (sym PO_2) showed no major intensity changes upon drug interaction.

The increase in the intensities of the bands at 1710 (G), 1662 (T), 1610 (A), and 1492 cm^{-1} (C), continued up to $r = 1/5$, indicating major zanamivir interaction with guanine, thymine, adenine, and cytosine. No major intensity changes were observed for the PO_2 symmetric and asymmetric bands (Fig. 2A).

At high drug concentration, $r = 1/2$, some reduction in the intensity ratios of the bands at 1710 (G), 1662 (T), 1610 (A), and 1492 cm^{-1} (C) were observed. The observed loss of intensity ratios were probably due to helix stabilization induced by the interaction of zanamivir with the DNA, no major intensity change was observed for phosphate group. A similar increase in the intensity ratios of several DNA vibrations was also observed when DNA was incubated with high concentrations of chlorophyll [17] and diethtylstlbestrol [28].

As concentration increased ($r = 1/1$), major increase in the intensities of adenine and thymine was observed which is indicative of major interaction with A-T base pairs (minor groove). Minor increase in the intensities of guanine and cytosine, asymmetric and symmetric phosphate are indicative of some degree of drug interactions with G-C bases and phosphate group.

3.2. FTIR spectra of zanamivir–RNA complexes

The Infrared spectra of zanamivir–RNA adducts with various molar ratios of zanamivir–RNA (phosphate) were recorded. The spectral changes (intensity or shifting) of several RNA in plane vibrations at 1704 cm^{-1} (G,U; mainly guanine), 1657 cm^{-1} (U, G, A, and C, mainly uracil), 1604 cm^{-1} (A), 1486 cm^{-1} (C), and 1241 cm^{-1} (PO_2 asymmetric stretch) [29–37] were monitored at different drug concentrations (Figs. 3 and 2B).

At $r = 1/20$, the intensity of the bands at 1704 cm^{-1} (G) (38%), 1657 cm^{-1} (U) (32%), 1604 cm^{-1} (A) (25%), 1486 cm^{-1} (C) (33%), and 1241 cm^{-1} (PO_2) (52%) decreased, respectively (relative to free RNA) (Fig. 2B). The major decrease in intensity was observed in the presence of zanamivir. The intensity variations were associated with the shift of the bands at 1704 (mainly G) to 1706 cm^{-1}, 1657 cm^{-1} (mainly U) to 1658 cm^{-1}, 1604 cm^{-1} (A) to 1613 cm^{-1}, 1486 cm^{-1} (C) to 1483 cm^{-1}, and 1241 cm^{-1} (PO_2 stretch) to 1238 cm^{-1} (Fig. 3). No major shifting was observed for uracil vibration. The observed spectral changes can be due to drug interaction (through OH and NH_2) with bases (mainly with adenine) and the backbone phosphate group (Figs. 3 and 2B).

As concentration increased ($r = 1/0$), no major change in intensity was observed (Fig. 2B). At high concentrations ($r = 1/5$, 1/2, 1/1), major intensity increase observed for uracil, guanine, adenine, cytosine, and to a lesser extent PO_2 vibrations. The intensity changes were accompanied with shifting of the bases and phosphate stretching vibrations (PO_2). The observed spectral changes can be due to drug interaction (through NH_2 and OH) with bases and the backbone phosphate group.

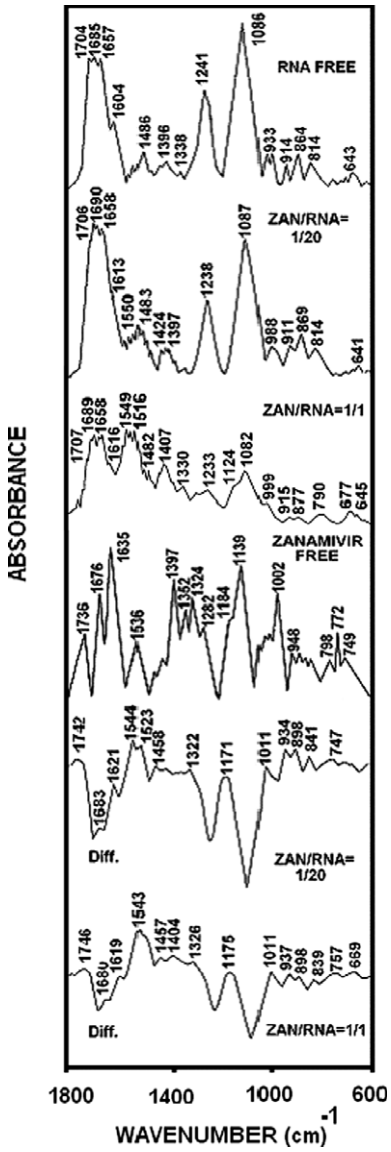

Fig. 3. FTIR spectra in the region of 1800–600 cm^{-1} for free yeast RNA, zanamivir, and zanamivir adducts in aqueous solution at pH 6.5–7.5 (top four spectra) and difference spectra obtained at various zanamivir–RNA (phosphate) molar ratios (bottom two spectra).

3.3. DNA and RNA conformation

In a complete B to A transition, the B-DNA marker bands are observed at 1710–1700 cm^{-1}, 1225–1240 cm^{-1}, 825–800 cm^{-1}, respectively, and a new band appears at about 870–860 cm^{-1} [22,37,38].

At higher zanamivir concentration ($r = 1/1$), a partial B to A-DNA transition occurred upon zanamivir adduct formation. Evidence for this comes from the shift of the sugar–phosphate band at 834 cm^{-1} (B-DNA marker) towards a lower frequency with major reduction in its intensity. In the difference spectra of zanamivir–DNA complexes ($r = 1$), the emergence of a new peak at about 820 cm^{-1} (A-DNA marker) is accompanied by major loss of the intensity of the band at 830 cm^{-1}. Similarly, the other B-DNA marker band at 1226 cm^{-1} shifted to 1238 cm^{-1} at $r = 1/1$. The observed spectral changes can be due to partial B to A transition upon zanamivir interaction.

The RNA remained in the A-conformation in zanamivir–RNA complexes (phosphodiester mode). The marker bands for A-RNA conformation at 864 and 814 cm^{-1} (due to ribose-phosphate vibrations), 1241 (PO_2 stretch) and 1704 cm^{-1} (G) did not show major shifting in the spectra of the zanamivir–RNA complexes, indicating of RNA remaining in A-conformation (Fig. 3). The minor intensity variations for RNA marker bands at 864 and 814 and 800 cm^{-1} are related to a perturbations of the sugar–phosphate backbone geometry upon drug interaction (Fig. 3), while RNA remaining in A-conformation [22,37–39].

3.4. Absorption spectra of zanamivir–DNA and zanamivir–RNA complexes

3.4.1. Stability of zanamivir–DNA, -RNA adducts

The calculation of the overall binding constants were carried out using UV spectroscopy as reported [40–42]. If the equilibrium for drug with DNA and RNA were established as:

$$\text{Drug} + \text{polynucleotide} <=> \text{Drug} : \text{polynucleotide}$$

$$K = [\text{Drug} : \text{polynucleotide}]/[\text{Drug}][\text{polynucleotide}]$$

polynucleotides = DNA or RNA

The double reciprocal plot of 1/[drug complexed] vs 1/[drug] is linear and the association binding constant (K) is calculated from the ratio of the intercept on the vertical coordinate axis to the slope (Fig. 4). Concentrations of complexed drug were determined by subtracting absorbance of uncomplexed DNA or RNA at 260 nm from those of the complexed DNA and RNA. Concentrations of drugs were determined by subtraction of complexed drug from total drug used for the experiment. Our data of 1/[drug complexed] almost proportionally increased as a function of 1/[free drug] (Fig. 4) and thus, the overall binding constants are estimated to be K(zanamivir–DNA) $= 1.30 \times 10^4\ \text{M}^{-1}$ and K(zanamivir–RNA) $= 1.38 \times 10^4\ \text{M}^{-1}$ complexes. Similar association constants were reported for DNA and RNA complexes with AZT [29,42], Taxol [43], chlorophyll, chlorophyllin [17,44,45], and biogenic polyamines [46]. The marked similarities of K values for drug–DNA and drug–RNA complexes observed here are indicative of a similar affinity of zanamivir for DNA and RNA complexation.

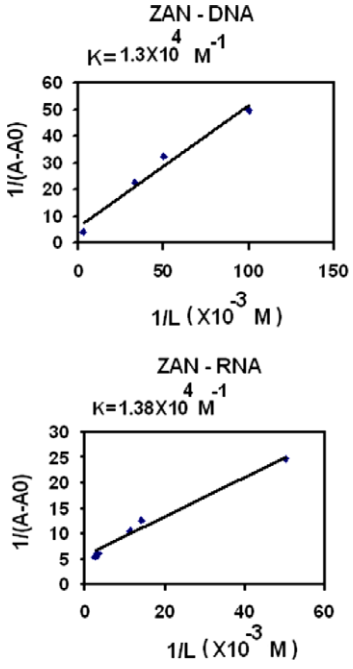

Fig. 4. The plot of 1/(A-A0) vs. 1/L for DNA and RNA and their drug complexes where A0 is the initial absorption of DNA or RNA (260 nm) and A is the recorded absorption at different zanamivir concentrations (L).

4. Conclusions

Zanamivir binds DNA and RNA at the major and minor grooves (H-bonding) double helices with little perturbations of the backbone phosphate group with overall binding constants of K(zanamivir–DNA) $= 1.30 \times 10^4\ \text{M}^{-1}$ and K(zanamivir–RNA) $= 1.38 \times 10^4\ \text{M}^{-1}$. The drug interaction induces a partial B to A-DNA transition, while RNA remains in A-conformation.

Acknowledgements

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