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RNA−Aspirin Interaction Studied by FTIR Difference Spectroscopy

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Aspirin is an old drug, which belongs to the nonsteroidal anti-inflammatory drugs. It is used extensively as a painkiller, and recently, it has been suggested to be effective against colorectal cancer. The aim of this study is to investigate the interaction of yeast RNA with aspirin in aqueous solution at physiological pH with drug/RNA(P) (P=phosphate) molar ratios of r = 1/80, 1/40, 1/20, 1/10, 1/4, 1/2, and 1. Fourier transform infrared (FTIR) difference spectroscopy is used to determine drug binding mode, sequence selectivity, and RNA secondary structure, as well as the structural variations of the aspirin−RNA complexes in aqueous solution. Spectroscopic evidence showed that at low drug concentration (r = 1/80), aspirin−RNA interaction is through the G-C and A-U base pairs and the backbone PO_2 group. Such interaction largely perturbs the G-C vibrations at 1698 and 1488 cm^{-1} and the A-U bands at 1654 and 1608 cm^{-1} as well as the phosphate antisymmetric stretch at 1244 cm^{-1}. At r = 1/40, minor structural changes occur for the ribose−phosphate backbone geometry with RNA remaining in the A-conformation. At r > 1/20, a partial helix destabilization occurs. The drug distributions around the double helix are about 50% G-C, 30% A-U, and 20% PO_2 groups. A comparison between aspirin−RNA and asprin−DNA complexes shows minor differences. The aspirin anion binding is via drug OCO and $COOCH_3$ groups.

Introduction

Aspirin belongs to the nonsteroidal anti-inflammatory drugs (NSAID), and it is used extensively as a painkiller. It exerts its anti-inflammatory effects through selective acetylation of serine 530 on prostaglandin H_2 synthase.[1] In recent years, aspirin was found to reduce the risk of heart attack and stroke. Aspirin is also suggested to be effective against colorectal cancer, although it has no effect on other types of cancers such as lung, breast, ovary, testis, lymphoma, and leukemia.[2−6] The exact mechanism by which aspirin exerts its antitumor activity is not clear. However, NSAID drugs are found to block the synthesis of prostaglandins long chain fatty acid compounds that have various functions, including stimulation of cell proliferation and suppression of immune reaction, both of which are linked to tumor progression. Recently, the structural basis of the aspirin activity inferred from the crystal structure of the inactivated prostaglandin H_2 synthase was reported.[1] If aspirin exhibits antitumor activity, its reaction with cell particles such as DNA, RNA, and protein is of major biological importance.

Recently, we have reported the effects of aspirin on the solution structure of calf-thymus DNA.[7] In this study, the aspirin−DNA binding site, sequence preference, and DNA secondary structure were determined using vibrational spectroscopy.[7] Vibrational spectroscopy is widely used to characterize the nature of drug−DNA interaction and to monitor the effects of various drugs on the solution structure of nucleic acids.[8] Recently, we used vibrational spectroscopy to analyze the nature of DNA−DES (diethylstilbestrol),[9] RNA−DES,[10] DNA−vitamin C,[11] DNA−cation,[12−15] and protein complexes.[16] We believe that vibrational spectroscopy can also be used here to examine the complex formation between RNA and aspirin and to elucidate the nature of this biologically important drug−RNA interaction. Our structural information provides, to our knowledge, the first spectroscopic evidence regarding aspirin−RNA interaction and should help to elucidate the nature of this biologically important complex formation at the molecular level.

In this work, we applied FTIR difference spectroscopy to examine the interaction of yeast RNA with aspirin in aqueous solution at pH 6−7 with aspirin to RNA(phosphate) molar ratios of r = 1/80 to 1. Spectroscopic evidence regarding aspirin−RNA complexation, drug binding mode, sequence selectivity, and RNA secondary structure is provided. Furthermore, comparisons were made between aspirin−RNA complexes and the aspirin−DNA adducts,[7] and the results are discussed here.

Materials and Methods.

Yeast RNA sodium salt was purchased from Sigma Chemical Co. and used as supplied. Aspirin was from Aldrich Chemical Co. and used without further purification.

Preparation of Stock Solutions. Sodium−RNA was dissolved to 2% w/w, 0.1 M RNA(phosphate) in 0.05 M NaCl and 1 mM sodium cacodylate solution (pH 7.30) at room temperature. A solution of 0.3−25 mM aspirin was also prepared in distilled water. In the final step, the appropriate amount of drug solution was added dropwise to RNA solution with constant stirring to give the desired aspirin/RNA(P) (P = phosphate) molar ratios of 1/80, 1/40, 1/20, 1/10, 1/4, 1/2, and 1 at a final RNA concentration of 1% w/w or 0.025 M RNA-(phosphate). Solution pH was adjusted to 6−7 with 0.1 M NaOH.

Infrared spectra were recorded on a Bomem DA3-0.02 Fourier transform infrared instrument equipped with a nitrogen-cooled HgCdTe detector and KBr beam splitter. Solution spectra were taken using AgBr windows with a resolution of 2 cm^{-1} and 100−500 scans. The H_2O subtraction was carried out as reported.[17] A good subtraction of water was achieved as shown by a flat base line around 2200 cm^{-1}, where the water combination mode is located (this portion of spectrum is not shown). The difference spectra [(RNA solution + aspirin solution) − (RNA solution)] were obtained using the RNA band at 966 cm^{-1} as an internal reference. This band due to the ribose

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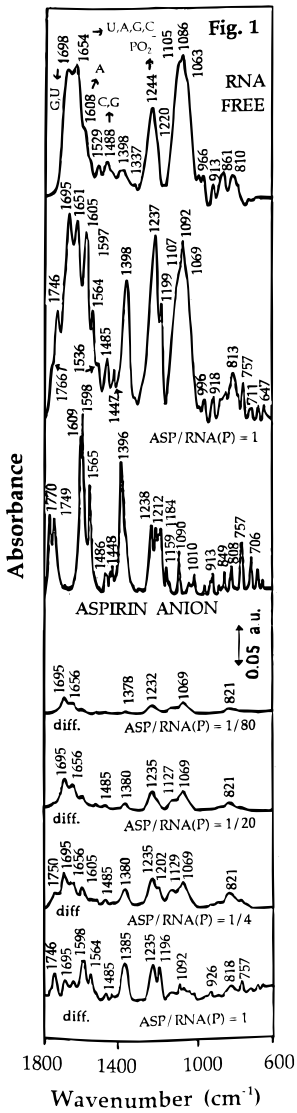

Figure 1. FTIR spectra (top three curves) and difference spectra [(RNA solution + aspirin solution) − (RNA solution)] (bottom four curves) for the uncomplexed yeast RNA and its aspirin complexes in aqueous solution at pH = 6−7 with different drug concentrations (aspirin to RNA(P) molar ratios) in the region of 1800−600 cm^{-1}.

C−C stretching modes exhibits no spectral changes (intensity or shifting) on drug−RNA interaction and is canceled upon spectral subtraction.

The calculated intensity ratios of several prominent RNA in-plane vibrations (normalized against the band at 966 cm^{-1}) were measured as a function of aspirin concentration with an error of ±5%. The results from calculated intensity ratios were used to quantify the amounts of drug−PO_2 and drug−base bindings. The detailed data manipulations for intensity ratio calculations are presented in our recent reports.[7,9−12]

Results and Discussion

RNA−Aspirin Complexes. At low drug concentration (r = 1/80), aspirin binding is through the G-C and A-U and the backbone PO_2 groups. Evidence for this comes from major intensity increase (20 to 50%) and shifting of several RNA in-plane vibrations[17−22] at 1698 (G,U), 1654 (U,G,A,C), 1608 (A), 1488 (C,G), and 1244 cm^{-1} (PO_2 antisymmetric stretch) (Figures 1 and 2). The positive derivative features at 1695, 1656, 1608, 1485, and 1232 cm^{-1} in the difference spectra of aspirin−RNA complexes are arising from a major increase in the intensity of several RNA vibrational frequencies upon drug complexation

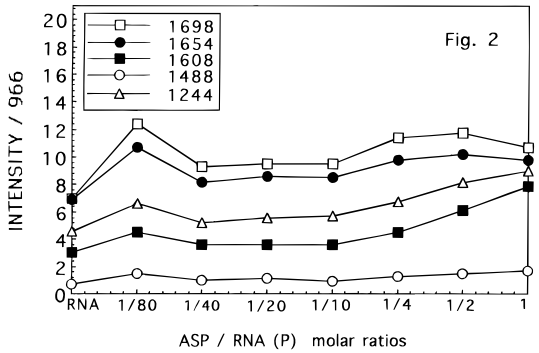

Figure 2. Calculated intensity ratios of several RNA in-plane vibrations at 1698 (G,U), 1654 (U, G, C,A), 1608 (A), 1488 (C,G), and 1244 cm^{-1} (PO_2) as a function of aspirin concentration (different aspirin to RNA(P) molar ratios).

(Figure 1, r = 1/80). The intensity increase of these vibrations is associated also with the shift of the bands at 1698 (mainly G) to 1695 cm^{-1}, whereas the bands at 1654, 1608, 1488, and 1244 cm^{-1} exhibited no shifting upon aspirin interaction. The observed spectral changes are related to a major drug interaction with the G-C base pairs (H-bonding to the guanine N-7 site via mediated H_2O) and to a lesser extent to A-U bases (H-bonding to uridine O-2 atom through H_2O) and to the backbone PO_2 groups. The calculated intensity ratios of RNA in-plane vibrations (related to A-U and G-C bases and the backbone PO_2 groups) as a function of aspirin concentration are presented in Figure 2. The intensity ratio measurements show 50% drug association with the G-C bases, 30% with A-U base pairs, and less than 20% with the backbone PO_2 (Figure 2).

At r = 1/40, a minor decrease in the intensity (20%) of the RNA bands at 1698, 1654, 1608, 1488, and 1244 cm^{-1} was observed (Figure 2). These intensity variations were also associated with the change in intensity of the RNA marker bands[23] at 810 cm^{-1} due to the ribose−phosphate stretching mode.[23] The observed spectral changes are due to a minor alteration of the sugar−phosphate backbone geometry upon drug interaction. However, since the main infrared marker bands for the A-conformation[23] are present at 813 cm^{-1} (sugar−phosphate vibration), 1242 cm^{-1} (PO_2 stretch) and 1695 cm^{-1} (G, U), the RNA remains in the A-family structure in these drug−RNA complexes (Figure 1). In a recent study, the effects of inorganic and organic anions on the solution structure of DNA were reported[24] and it was shown that organic anions stabilize the formation of a certain DNA conformation over other structures. It has been also shown that aspirin induces a partial reduction of B-DNA structure in favor of A-DNA in several aspirin−DNA complexes.[7] However, apart from minor alterations of the ribose−phosphate backbone geometry, it seems the aspirin anion stabilizes the A-conformation of RNA in these drug−RNA complexes.

At high drug concentration (r > 1/10), a minor helix destabilization occurs. Evidence for this comes from an increase in the intensity (30%) of the RNA bands at 1698, 1654, 1608, 1488, and 1244 cm^{-1} (Figure 2). The strong positive derivative features observed at 1695, 1656, 1605, 1485, and 1235 cm^{-1} in the difference spectra of the aspirin−RNA complexes are consistent with the intensity increase of RNA in-plane vibrations (Figure 1, r = 1/4). The weak positive derivative features at 1750, 1380, 1202, and 1129 cm^{-1} in the difference spectra arise from aspirin vibrations, and they are not related to the RNA molecule (Figure 1, r = 1/4). The partial helix opening increases the chance of aspirin binding to additional donor sites available on local helix melting (such as guanine, cytosine, and uridine carbonyl groups and guanine, adenine, and cytosine NH_2

groups). Evidence for this comes from a major shift of the band at 1654 (U,G,C,A) to 1651 cm^{-1}, 1608 (A) to 1605 cm^{-1}, 1488 (C,G) to 1485 cm^{-1}, and 1244 (PO_2 stretch) to 1237 cm^{-1} (Figure 1, $r = 1$). Similar local helix opening was observed for calf-thymus DNA in the presence of high aspirin concentration.[7] It should be noted that the helix melting by thermal denaturation or by cation coordination also causes a major increase in the intensity of several DNA or RNA in-plane vibrations.[8,14,15] However, at $r = 1$, no major change of intensity was observed for RNA in-plane vibrations, although spectral shifting was continued (Figure 2). The weak positive features at 1695, 1651, and 1485 cm^{-1} in the difference spectra of drug−RNA complexes are related to minor intensity variations of RNA in-plane vibrations, while the strong positive features at 1748, 1599, 1564, 1385, 1196, and 1015 cm^{-1} are due to the aspirin anion vibrations (Figure 1, $r = 1$).

Additional evidence for aspirin−RNA complexation comes from major spectral shifting of the aspirin anion vibrational frequencies upon RNA interaction. Several strong vibrations at 1770 (C═O stretch), 1749 (C═O stretch), 1609 (C═C stretch), 1598 (OCO antisymmetric stretch), 1565 (C═C stretch), 1398 (OCO symmetric stretch) and 1238, 1212, and 1184 cm^{-1} (C−O and C−C stretching modes) in the spectrum of the free aspirin anion[7] exhibited spectral shifting toward lower frequencies upon RNA interaction (Figure 1). The observed spectral shifts are the results of a major aspirin−RNA interaction via drug OCO and $COOCH_3$ donor groups (directly or indirectly through water molecules).

Comparison between Aspirin−RNA and Aspirin−DNA Complexes. On the basis of our vibrational spectroscopic results presented for aspirin−DNA[7] and aspirin−RNA complexes for the first time, it was clearly shown that aspirin binding to DNA was mainly through the A-T base pairs and the backbone PO_2 group at low drug concentration and extended to the G-C bases at high aspirin content.[7] The aspirin complexation resulted in a major reduction of B-DNA structure in favor of A-DNA with a partial helix opening.[7] However, the aspirin−RNA interaction occurs through the G-C, A-U, and the backbone PO_2 groups at low drug concentration, whereas at high drug content, a minor helix destabilization is observed. Although aspirin−RNA interaction causes minor alterations of the ribose−phosphate backbone geometry, RNA remains in the A-family structure in the these aspirin−RNA complexes.

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Abbreviations

Asp	aspirin
NSAID	nonsteroidal anti-inflammatory drug
DES	diethylstilbestrol
FTIR	Fourier transform infrared
A	adenine
C	cytosine
G	guanine
T	thymine
U	uridine
r	drug/RNA(phosphate) molar ratio

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