RNA-Diethylstilbestrol Interaction Studied by Fourier Transform Infrared Difference Spectroscopy

Jean-François Neault and Heidar-Ali Tajmir-Riahi
From the Department of Chemistry-Biology, University of Québec at Trois-Rivières, C.P. 500 TR, Québec, Canada G9A 5H7

Diethylstilbestrol (DES), a synthetic estrogen, is known to be a carcinogen in human and in animals. This study was designed to examine the interaction of DES with yeast RNA in aqueous solution at physiological pH with drug/RNA-phosphate (P) molar ratios of 1/80, 1/40, 1/20, 1/10, 1/4, and 1/2. Fourier transform infrared (FTIR) difference spectroscopy was used to determine the drug binding mode, the binding constant, the sequence selectivity, and RNA secondary structure in the RNA-DES complexes.

Spectroscopic evidence showed that at low drug concentration (1/80 and 1/40), DES is intercalating through both Gua-Cyt and Ade-Urd base pairs with minor interaction with the backbone PO2 group (external binding). The calculated binding constant of K ≈ 8.5 × 10^4 M^-1 at a drug concentration of 3.12 × 10^-4 M shows that DES is a weaker intercalator than those of the methylene blue, acridine orange, and ethidium bromide. At high drug content (r > 1/40, where r represents the DES/RNA-phosphate molar ratio), a partial helix destabilization occurs with no alteration of RNA conformation upon drug complexation. However, a comparison with DNA-DES complexes showed that drug intercalation causes major reduction of the B-DNA structure in favor of A-DNA with no participation of the backbone PO2 group in the DES-DNA complexation.

DES (see Structure 1), a synthetic estrogen, is known to be a carcinogen both in human and in animals (1, 2). It was used worldwide from the 1940s until 1970s to prevent miscarriages in women, while at the same time, it was found to cause cancer in experimental animals. Although DES application has been banned for pregnancy, it remains in use for other clinical purposes such as estrogen replacement therapy for hormone deficiency. The DES-induced carcinogenesis has been considered to be due to the high hormonal potency of this synthetic estrogen and possible DES metabolites (6).

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† To whom correspondence should be addressed; Tel.: 819-376-5077 (ext. 3321); Fax: 819-376-5057; E-mail: Tajmir@uqtr.uquebec.ca.

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The intensity ratios of several RNA in-plane vibrations related to Ade-Urd, Gua-Cyt, and the backbone PO$_2$ groups were measured as a function of drug concentration with error of $\pm$ 5%. These intensity ratio variations were used to quantitatively measure the amounts of drug base and drug-PO$_2$ bindings. The detailed intensity ratio measurements and spectral manipulations are described in our previous publication (16).

The calculation of the binding constant was also carried out as reported for other drug complexes with DNA, RNA, and mononucleotides (20–23). By assuming that there is only one type of interaction (intercalation) between drug and RNA molecule, Equations 1 and 2 can be established.

$$\text{RNA} + \text{DES} \leftrightarrow \text{RNA-DES} \quad K \quad \text{(Eq. 1)}$$

$$K = \frac{[\text{RNA-DES}]}{[\text{RNA}][\text{DES}]} \quad \text{(Eq. 2)}$$

The relative intensities for the band at 1698 cm$^{-1}$ (mainly Gua), at 1608 cm$^{-1}$ (mainly Ade), and at 1244 cm$^{-1}$ (backbone PO$_2$) were calculated for each drug concentration. The calculated intensities were used as a function of drug concentration to estimate the $K$ for guanine, $K(A)$ for adenine, and $K(PO_2)$ for the phosphate group. The overall association constant ($K$) estimated for the above equation was $8.5 \times 10^4$ M$^{-1}$ at DES concentration of $3.12 \times 10^{-4}$ M.

RESULTS AND DISCUSSION

RNA-DES Complexes—At low drug concentration ($r = 1/80$ and 1/40), DES (Structure 1) intercalates in the Gua-Cyt and Ade-Urd-rich regions. Evidence for this comes from minor intensity increase (10%) of the RNA in-plane vibrations at 1698 cm$^{-1}$, where the H$_2$O combination mode is located (19). The difference spectra [(RNA solution + DES solution) - (RNA solution)] were produced, using the RNA band at 913 cm$^{-1}$ as internal reference. This band, due to ribose-phosphate vibration exhibits no spectral changes (intensity or shifting) upon drug complexation, and it is cancelled on spectral subtraction. The difference spectra contain several positive and negative derivative features in the region of 1800–600 cm$^{-1}$, whose amplitudes are less than 20% of the original peaks, with an estimated error of $\pm$ 5% (absorbance). Several positive features in the difference spectra of DES-RNA complexes are coming from DES vibrations and are not due to RNA vibrational mode (properly labeled).

A comparison with the infrared spectra of other strong DNA and RNA intercalators, such as ethidium bromide, acridine orange, and methylene blue (recorded in our laboratory), showed that DES is a weaker intercalator than these pigments. The calculated binding constants for DNA-pigment and RNA-
pigment complexes are ranging from $10^9$ m$^{-1}$ to $10^6$ m$^{-1}$ (21–23, 35–38). These pigments are intercalated in both Gua-Cyt, Ade-Thy, or Ade-Urd-rich regions with no major sequence preference and with minor drug-phosphate interaction (external binding), similar to RNA-DES complexes investigated here.

At $r > 1/40$, DES interaction causes a partial helix destabilization. Evidence for this comes from major increase (20%) in the intensity of RNA bands at 1698, 1654, 1608, and 1244 cm$^{-1}$ (Fig. 2, $r = 1/20$). A similar increase of intensity was observed for several DNA or RNA in-plane vibrations upon thermal denaturation and acid fixation (16, 23). The partial helix opening increases the chance of drug binding (externally) to different RNA donor sites that are available on local helix melting. At $r > 1/20$, some decrease in the intensity (20–30%) of several RNA bands at 1698, 1654, 1608, and 1244 cm$^{-1}$ (backbone PO$_4$ stretch) as a function of drug concentration (different DES/RNA-phosphate (RNA/P)) molar ratios).

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