DNA-HARMALOL INTERACTION, THE EFFECTS OF HARMALOL ON THE SOLUTION STRUCTURE OF CALF-THYMUS DNA STUDIED BY FTIR SPECTROSCOPY

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ABSTRACT

The interaction of haromalol with calf-thymus DNA was investigated at physiological pH with drug/DNA (phosphate) molar ratio(r) of 1/40. Fourier transform infrared difference spectroscopy were used to establish correlations between spectral changes and drug binding mode, sequence selectivity, DNA conformation and structural properties of haromalol-DNA complexes in aqueous solution. Spectroscopic results indicated that haromalol is a weak intercalator with affinity for A-T rich regions. At low drug concentration(r=1/40), the A-T region is the main target of drug intercalation.

Key words: Calf-thymus DNA, Harmalol, FTIR

INTRODUCTION

Harmalol which is isolated from peganum harmala L. as well as Banisteria Caapi spruce (1-2) has been shown to play an important role in human cancer (3). The exact mechanism by which Harmalol exerts its anticancer activity is not clear and investigation of its interaction with cell particles such as nucleic acids, DNA polymerases and proteins is of great biological importance. Vibrational spectroscopy (infra-red) is often used to characterize the nature of drug/DNA interaction and to monitor the effects of various drugs on DNA structure (4-6). Recently, vibrational spectroscopy has been applied to analyze the nature of vitamin C, aspirin, chlorophyllin, dihydroxyisobenzol (DES) complexes with DNA (7-10). Our interest on biochemical activities of harmalol prompted us to investigate the drug binding sites, sequence specificity, helical stability and DNA secondary structure of DNA-harmolol interactions in aqueous solution by FTIR spectroscopy. In this communication, the interaction of harmalol with calf thymus DNA with molar ratio of r=1/40 in water/ethanol (75/25 v/v) solution of pH=6-7 by FTIR spectroscopic technique is determined.

MATERIALS AND METHODS

Chemicals: Highly polymerized calf-thymus DNA sodium salt (7% sodium content) was from Sigma Chemical Co. and used as supplied. Harmalol was purchased from Fluka Chemical Co. and used without further purification.

Preparation of stock solution: sodium DNA solution was prepared in 4% w/v (0.1M phosphate) in 0.1M NaCl solution at 5°C for 24h with occasional stirring to ensure formation of a homogeneous solution. A solution of harmalol was also prepared in water/ethanol solution (75/25 v/v). Mixtures of drug and DNA were prepared by adding harmalol solution dropwise to DNA solution with constant stirring to give the desired drug/DNA molar ratios of 1/40. Solution pH was kept near 7.5 to 6.5 by NaOH solution (0.1 M). The IR spectra were recorded 4 h after initial mixing of drug and DNA solution on a BOMEM MB series Fourier transform infrared spectrometer with a nitrogen-cooled HgCdTe detector and a KBr beam splitter. Solution spectra were taken using CaF2 windows with a resolution of 2 cm⁻¹ and 400-600 scans. Water subtraction was carried out as reported (11). A good subtraction was achieved as shown by a flat baseline around 2200 cm⁻¹, where the water combination mode is located.

RESULTS AND DISCUSSION

The FTIR spectra related to harmalol interaction with DNA are tabulated in Table 1. The IR spectrum of the free harmalol in aqueous solution contains several important bands with strong and medium intensities at 1723 and 1659
Figure 1. FTIR spectrum of Harmalol

Figure 2. FTIR spectrum of DNA
DNA-harmalol interaction

Figure 3. FTIR spectrum of DNA-Harmalol complex

1640 cm\(^{-1}\) (C=O, C-O and C-N stretching) and 1640 cm\(^{-1}\) (assigned to the N-H and H\(_2\)O bands) (12). The absorption bands at about 1450-1550 cm\(^{-1}\) in the harmalol spectrum corresponds to C=N vibrations. The medium and weak absorption bands at 1040, 1142, 1257, 1331, 1380 and 1450 cm\(^{-1}\) are assigned to O-H and C-H deformations, C-C and C-O stretching.

Drug-DNA complexation: At low harmalol concentration (r = 1/40), the band at 1715 cm\(^{-1}\) (G,T) in the DNA spectrum shifted towards a lower frequency at 1710 cm\(^{-1}\) in the DNA harmalol spectrum. No intensity variation is observed for this frequency upon drug interaction. This is indicative of no major harmalol interaction via G-C base pairs at this stage. However, major intensity increase was observed in the infrared bands of 1661 cm\(^{-1}\) (mainly thymine) 1609 cm\(^{-1}\) (mainly adenine) and at 1225 cm\(^{-1}\) (PO\(_2\) antisymmetric stretching (Table 1). The intensity increase of these vibrations is also associated with the shift of the bands of 1661 to 1667 cm\(^{-1}\), 1609 to 1603 cm\(^{-1}\) and 1225 to 1220 cm\(^{-1}\). The observed spectral changes are due to the drug-DNA interaction via backbone phosphate group and thymine O-2 and adenine N-7 atoms (with N-H and O-H groups of harmalol) that are not normally involved in Watson-Crick hydrogen bonding network. Such interaction does not bring about helix destabilization (Figures 1, 2, 3) (8-10). In conclusion, at low drug concentration (r = 1/40),

<table>
<thead>
<tr>
<th>Harmalol / DNA</th>
<th>Assignments</th>
</tr>
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<tbody>
<tr>
<td>DNA 1/40</td>
<td>R</td>
</tr>
<tr>
<td>1710 s</td>
<td>G, T</td>
</tr>
<tr>
<td>1661 w</td>
<td>T, G, A, C</td>
</tr>
<tr>
<td>1659 s</td>
<td>Harmalol</td>
</tr>
<tr>
<td>1643 s</td>
<td>T</td>
</tr>
<tr>
<td>1645 s</td>
<td>H(_2)O</td>
</tr>
<tr>
<td>1603 s</td>
<td>A</td>
</tr>
<tr>
<td>1488 w</td>
<td>A, G</td>
</tr>
<tr>
<td>1370 w</td>
<td>T, G, A, C</td>
</tr>
<tr>
<td>1275 w</td>
<td>A, C</td>
</tr>
<tr>
<td>1220 s</td>
<td>PO(_2)</td>
</tr>
<tr>
<td>1086 m</td>
<td>PO(_2)</td>
</tr>
<tr>
<td>1045 s</td>
<td>T, G, A, C</td>
</tr>
</tbody>
</table>

the interaction of harmalol with calf thymus DNA results in minor perturbations of the backbone phosphate group and the A-T sites. Similar changes have been reported in aspirin-DNA complexation at the same concentration.

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REFERENCES