DNA-chlorpheniramine interaction studied by spectroscopic techniques

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Received 20 May 1997; revised 21 July 1997; accepted 21 July 1997

Abstract

It was previously studied that the antihistaminic chlorpheniramine elicits a biphasic response on cell growth and regulates polyamine metabolism, as described for polyamines. In part, polyamine effects on macromolecular synthesis and cell growth are attributed to nucleic acid:polyamine interactions. In this work, we have tested the hypothesis of a DNA:chlorpheniramine interaction, using fluorometry, FTIR and Raman spectroscopic techniques. The results indicate that DNA:chlorpheniramine interaction occurs inducing conformational changes in the macromolecule by affecting both phosphodiester bonds and bases. Results open new perspectives for characterization of action mechanisms of natural or synthetic diamines with pharmacological or physiological importance. © 1998 Elsevier Science B.V.

Keywords: Chlorpheniramine; DNA structure; Polyamine; Laser Raman; FTIR

1. Introduction

Chlorpheniramine (gamma-(4-chlorphenyl)-N,N-dimethyl-2-pyridinopropanoamine or CPA) is a 1,4-diamine described as antagonist of the histamine receptor, subtype H1 of mammalian cells [1]. As histamine and ornithine-derived polyamines (putrescine, spermidine and spermine), CPA is an amphipathic molecule containing a charged amino group at physiological pH and a hydrophobic skeleton. Intracellular concentrations of polyamines control synthesis and stability of the enzyme limitant for polyamine biosynthesis, ornithine decarboxylase (ODC, EC 4.1.1.17) [2,3]. Previously, we have described that micromolar concentrations of histamine, CPA, and other 1,4-diamines having some structural features in common with ornithine-derived polyamines, actively accumulate inside the cell and also regulate the synthesis of mammalian ornithine decarboxylase [4]. The effect of CPA on ODC expression was observed working with tumor cell lines in culture, as well as with tumor-bearing mice in vivo [5,6]. After characterization of the molecular effect of CPA on mammalian ODC expression, we concluded that CPA alters the initiation of the ODC mRNA translation [5,6]. Some reports indicate that the polyamine spermidine also inhibits the initiation of the mammalian ODC mRNA translation by stabilizing the complex secondary structure of its 5′-untranslated region [7,8]; this is one of the most important
mechanisms for regulation of mammalian ODC expression [9,10].

Alteration of nucleic acid structure by ornithine-derived polyamines is shown, specially in the case of spermine and spermidine [11,12]. Spermine (Spi) interacts with the B-DNA groves inducing DNA bending and condensation of the macromolecule, and favoring B/A and B/Z transitions on appropriate DNA sequences [13,14]. Thus, it is proven that ornithine-derived polyamines are essential for maintaining macromolecular synthesis rate and fidelity; however, an excess of intracellular polyamines is lethal for the cell [15,16].

Cell growth also presents a biphasic response to CPA ([6], and unpublished experiments). Since the antiproliferative effect of CPA observed in vivo and in cultured cells cannot be fully explained on the base of its inhibitory effects on polyamine biosynthesis, we postulate the occurrence of nucleic acid:CPA interactions. In this report, we demonstrate that CPA induces changes in DNA conformation. The interaction seems to be topologically different to that described for spermine.

2. Materials and methods

CPA [(+)-chlorpheniramne, maleate salt], calf-thymus (CT-DNA) and salmon sperm DNAs (sodium salts) were purchased from Sigma Química (Spain); pBluescript KSII was from Stratagene (USA). Quenching of fluorescence of DNA:EtBr complexes was followed by the method described by LePecq and Paolelli [17], using a Hitachi spectrofluorophotometer (excitation wavelength 525 nm, emission wavelength 590 nm). Salmon sperm DNA solution (50 μg/ml) in 10 mM Tris-HCl, 50 mM NaCl, and an EtBr solution (0.5 μg/ml) in 5 mM Tris, 0.5 mM EDTA, both at pH 8 were used. CPA or Spi were added at different concentrations. pBluescript KS DNA obtained by the alkaline lysis method from transformed XL-Blue cells [18] was used for electrophoresis in agarose gels of DNA treated or not with variable amounts of CPA. Equal quantities of the plasmid were dissolved in a TE solution containing CPA. After 10 min, samples were loaded in a 9 × 6 cm agarose gel (1%) and electrophoresed in TAE medium (40 mM Tris-acetate, 1 mM EDTA, pH 8). After electrophoresis, gel was stained with ethidium bromide (1 μg/ml) for 10 min at room temperature [16]. Visualization of the bands was carried out with a transilluminator and a 265 nm lamp. For Raman and FT-IR experiments, CT-DNA was dissolved to 4% w/W in 0.1 M NaCl solution at neutral pH. Raman spectra at room temperature were recorded using a Jobin Yvon Ramanor U1000 spectrometer. Excitation radiation wavenumber at 514.5 nm was used as generated by a Spectra Physics argon-ion laser working at 500 mw as described by Neault et al. [19]. A minimum of 15 scans were accumulated to improve the signal-to-noise ratio. The best resolution obtained was 1 cm⁻¹. Raman spectra for DNA and DNA:CPA complexes were minimally smoothed. Infrared absorption spectra over probe energies of 1350–900 cm⁻¹ were recorded at room temperature in a Bruker Equinox 55 Fourier transform spectrophotometer purged with argon gas. Spectral resolution of 2 cm⁻¹ were reached and a minimum of 100 scans were accumulated for each sample.

3. Results and discussion

As mentioned above, during CPA studies on polyamine metabolism and ODC expression, we observed that CPA could mimick some polyamine effects at different levels: alteration of intracellular polyamine pools, inhibition of ODC translation, cell growth modulation, and so on. Thus, the possibility of similarities between the interactions of both 1,4-diamines, CPA and Spi, with DNA needed to be tested. Interaction of spermine with DNA is a well studied topic. Several physical chemical studies and theoretical models have been described indicating that spermine seems to interact with the DNA groves [11–14,20]. The interaction is easily observed by following the quenching of fluorescence caused by Spi on the DNA:EtBr complex. Micromolar concentrations of spermine causes a reduction in the fluorescence due to the interaction of EtBr with DNA [17]. These reported data for spermine were confirmed in the present work (Fig. 1A). In parallel, we followed the quenching of fluorescence caused by CPA on DNA:EtBr, between 1.7 μM and 50 mM CPA. DNA:EtBr complexes gained fluorescence when CPA was present at concentrations lower than 1 mM. CPA
did not exhibit fluorescence properties at concentrations used in this work. No increase in fluorescence could be detected in DNA:CPA solutions. CPA was not able to increase the fluorescence of the EtBr solution in the absence of DNA either. These results indicate that DNA:CPA interaction should be different to those reported for DNA:Spi complexes. A reduction in the DNA:EtBr fluorescence caused by CPA could only be observed at antihistaminic concentrations higher than 5 mM.

The same quantity of the plasmid pBSKSII pre-treated with different quantities of CPA were differentially stained by EtBr after electrophoresis, as shown in Fig. 1B. An increase in fluorescence was observed in the plasmid sample pretreated with 1 mM CPA respect to control DNA. As observed during measurements of DNA:EtBr fluorescence, signals of EtBr-stained DNA were lower when DNA samples had been treated with higher CPA concentrations. Combined results suggest that submillimolar concent-

![Fig. 1](image)

Fig. 1. (A) Quenching of EtBr:DNA complex fluorescence after addition of different concentrations of CPA (●) or Spi (○). The insert graph shows results obtained with CPA at the millimolar range. (B) The picture shows a negative image of the results obtained after EtBr (1 μg/ml) staining of an electrophoresis gel carried out with 1 μg of native pBluescript KS per lane pretreated with 0 (lane 1), 1 mM (lane 2), or 40 mM (lane 3) CPA.

![Fig. 2](image)

Fig. 2. Raman spectra of CPA (0.1 M), DNA:CPA complexes at 1/100 and 1/1000 molar ratios (500 and 50 μM CPA, respectively), and DNA alone (4% w/w) in the region of 1800–500 cm⁻¹.

![Fig. 2](image)

trations of CPA must alter the base conformation so that it potentiates the interaction between the intercalating dye and bases. At higher concentrations CPA could interfere with the accessibility of the dye to the DNA core.

In order to gain further information on the modifications of the DNA structure caused by CPA, we have registered Raman spectra of CT-DNA with different amounts of the antihistaminic. Fig. 2 displays the spectra of CPA/DNA:CPA solutions with ratios 1000:1 and 100:1 (corresponding to 50 and 500 μM CPA, respectively) together with those of free DNA (4% w/w) and CPA (0.1 M). No dramatic changes are observed in the spectra corresponding to DNA:CPA solutions with respect to the free DNA spectrum. Specific CPA bands did not appear either, as a consequence of its low concentrations. However, after more careful focusing and measuring of specific
bands, important shifts of marker bands were observed. The band at 837 cm$^{-1}$ in free DNA has been assigned to the antisymmetrical O–P–O stretching vibration [19,21]. Submillimolar concentrations of CPA downshifted this band by 5–6 cm$^{-1}$. In addition, a strong band around 1094 cm$^{-1}$ upshifts by 1–2 cm$^{-1}$. It has been clearly assigned to the PO$_2^-$ symmetrical stretching vibration [21]. These results are compatible with a relaxation of the phosphodiester bonds; further, as a consequence, the PO$_2^-$ bonds would become slightly stronger by withdrawing some of the peripheric charge excess, so that its related frequency would upshift. Thus, results indicate that interaction of DNA with CPA must alter the phosphate backbone of DNA. The observed changes are compatible with an increase in the distance between two contiguous residues and/or with a tendency to a widening of the helix.

The spectrum of free DNA shows a series of bands between 1600–1200 and 850–650 cm$^{-1}$ that have been assigned to base vibrations [21]. When CPA is added at different concentrations, most of these bands shift by 1–4 cm$^{-1}$, the remaining bands assigned to bases remain unperturbed. Interactions between CPA moieties and either purine or pyrimidine residues of the bases could explain the observed shifts. More specifically, bands at 1320 and 682 cm$^{-1}$ were observed in the Raman spectrum at 1000:1 DNA:CPA ratio (50 μM CPA), which were measured at 1317 and 679 cm$^{-1}$ in free DNA, respectively. They have been assigned to G residues (Fig. 2). Other three bands related to A,T and C residues show smaller shifts. Moreover, when using a 10:1 DNA:CPA ratio (5 mM CPA, spectrum not shown), free CT-DNA Raman frequencies 1377 (T,A,G), 1256 (C,T), 784 (C,T) and 729 (A) cm$^{-1}$ were measured at 1378, 1259, 788 and 732 cm$^{-1}$, respectively, and two additional bands related to guanine vibrations upshift by 1 cm$^{-1}$. Under the used conditions, results indicate that submillimolar concentrations of CPA seem mostly to affect phosphodiester bonds and guanine residues, while higher concentrations could involve both puric and pyrimidinic bases.

The infrared spectra of CT-DNA in the presence or absence of CPA were also analyzed (see Fig. 3) to support the Raman data discussed above. We used the same antihistaminic concentrations to compare both sets of spectroscopic results. Strong absorptions arose from water molecules only permitting us to examine the 1350–900 cm$^{-1}$ region; nevertheless, some trends can be clearly inferred. The band measured at 1225 cm$^{-1}$ in the infrared spectrum of free DNA increases its intensity, in relation to the other bands, when CPA is added. As Fig. 3 shows, this increase seems to be concentration-dependent. In addition, the same band appears at 1229 and 1231 cm$^{-1}$ for 50 and 500 μM CPA solutions, respectively. This band has been assigned to the phosphate antisymmetric stretching vibrations of DNA double-helical chains, and it is considered as one of the infrared marker bands of a B conformation. The band at 1087 cm$^{-1}$ has been assigned to a furanose ring vibration, while the rest of the measured bands have been related to different base vibrations. All of them up-
shift by 1–3 cm⁻¹ when CPA is added. These spectra changes are fully compatible with those observed in the Raman spectra. Thus a major CPA-DNA interaction at the phosphate backbone is confirmed. The infrared measurements indicate that bases and furanose rings are also involved, although not so strongly, in this interaction.

These results support that, in fact, CPA is able to interact with nucleic acids, as previously suggested by experimental observations of the action of CPA on whole cells and animals. DNA:CPA interaction must be topologically different of that described for Spi. However, as deduced from the different experimental approaches, DNA:CPA interaction must be topologically different of that described for Spi/DNA [13,14].

Macromolecular synthesis and cell growth exhibit a biphasic response to polyamine concentrations [3,16]. We have also observed on different eukaryotic models that CPA at low concentrations (lower than 50 μM) stimulate cell proliferation, but it is cytotoxic at higher concentrations ([6] and unpublished experiments). CPA was able to reduce the tumor growth in vivo and to act as a potent cytotoxic compound on tumor cell lines in culture. Even when DNA interaction with Spi and CPA do not seem to have exactly the same nature, both amphipathic diamines induce some similar effects on polyamine metabolism and cell growth [4]. Thus, our results should encourage both biochemist and physics-chemists to work together on the characterization of the interactions and effects of other amphipathic amines on DNA conformations, some of them being closely related to cell growth, and having high relevance in molecular physiology (i.e.: histamine) [1,22–25].

Acknowledgements

Thanks to Dr. Núñez de Castro and Dr. López-Herrera for valuable advice and comments. Thanks are due to Dr. Claros for help during computer work. This research was supported by Grant PB94-1474 (DGICYT, Spain), and funds from P.A.I. (#3218), Junta de Andalucía, Spain. FTIR spectrophotometer was acquired with the financial support from Project CICYT IN95-0199 and University of Málaga.