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Curcumin Complex-DNA Interaction Studied By Fourier Transform Infrared Spectroscopy

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Abstract : Curcumin is a non toxic, a dietary natural polyphenol known for its potent anti-inflammatory, antioxidant, antimutagenic and anticarcinogenic effects. The interaction of curcumin Complex(Curcumin and metal ions) with DNA is widely used in clinical treatment of a variety of neoplastic diseases, but its therapeutic effects is not fully understood. We here report the binding mechanism and structural properties of curcumin with DNA considered as a therapeutic target. The interaction of DNA with curcumin is investigated in aqueous solution at physiological pH with durg/DNA molar ratios of r=1:40, 1:20, 1:10, 1:5 and 1:2. Fourier Transform Infrared(FT-IR) and UV-absorption spectroscopy are used to determine the secondary structure of drug and DNA, drug binding sites, binding constant, as well as the structural variations of curcumin-DNA complexes in aqueous solution. Our spectroscopic result showed that interaction occurred mainly through G-C ,A-T base pairs and major interaction with phosphate groups with overall binding constant of K= 2.95×10^5 M^{-1}. Curcumin complexes maybe induced biopolymer conformational changes with DNA in the presence of metal ions.
Keywords: DNA**,** Curcumin; FT-IR spectroscopy.

Introduction

Structural features of nucleic acids in biological assemblies are often interpreted in relation to the high resolution X-ray structures obtained from oligonucleotide single crystals. To investigate native DNA structures directly and to advance the understanding of the biological roles of DNA in supramolecular assemblies, sensitive probes applicable to aqueous solutions are required. The FT-IR spectroscopy can provide definitive information about covalent bonding configuration, electrostatic, hydrophobic and hydrogen bonding interaction.

In this chapter the study of calf-thymus DNA in H_2O and curcumin in ethanol are presented. The FT-IR spectroscopy is used as a probe of the dynamics of Calf-thymus DNA and Curcumin by monitoring the hydrogen bonding network.

Results and Discussion

The FT-IR spectra of DNA and curcumin in aqueous solution between 3500-600 are presented in fig(i) and (ii) respectively. In the subsequent discussion the FT-IR spectra has been subdivided into different distict wave number region which provide information on different aspects of DNA conformation.

In-plane double bond stretching vibration of bases

The principal bands in the 2000-1500cm-1 region are due to C=C and C=O stretching. Carbonyl stretching is one of the easiest absorptions to recognize in an infrared spectrum. It is usually the most intense band in the spectrum, and depending on the type of C=O bond, occurs in the 1830-1650.

The 1800-1550cm-1 spectral region is considered as being sensitive to base pairing and so base stacking. It has been shown by X-ray diffraction that the B-form is a double helix structure with Watson-Crick base pairing. In polynucleotides, the existence of transition dipole coupling for C=O stretching vibrations was first pointed out by Tsuboi et al. As the base pair stacking is changed under the B to Z transition, the dipole interaction is modified, as can be detected by shifts of the absorption bands.(1)

The band observed at 1710cm-1 in H2O solution, in-plane stretching vibrations of double bond of guanine (C7=N) located at the major groove(2,3). In the interval 1600-1700cm-1 where thymine carbonyl(C2=O and C4=O) and ring double-bond (C5=C6) stretching vibrations are the expected major contributors. Assignment of the bands to specific C=O and C=C stretching coordinates is not trivial, but is important for interpreting hydrogen-bonding interactions in the duplex structure. An absorption at 1649 cm-1 , which is due either to an in-plane ring vibration of cytosine or C4=O4 of thymine or both, and the band at 1610 cm-1 arising from a ring in-plane vibration of adenine, thymine and cytosine.(2,4)

The 1610cm-1 band in FT-IR spectrum of DNA in H2O has been assigned to 2C=O stretching and NH2 scissoring vibration.(2,4)

The 1530cm-1 band of DNA has been assigned to vibration localized in the imidazolium ring of adenine. This band 1530cm-1 may be responsive to hydrogen-bond formation not only at N7, but also at N1 and/or N3.(5)(DNA-Ag)

The infrared band located at 1492cm-1 in H2O solution has been assigned to a vibration involving a large displacement of N7 and C8 atoms of purine. In the B-DNA structure it involves mainly cytosines with a weak contribution of guanines .(5,6)

The weak IR band near 1422cm-1 in H2O solution has been assigned to C2'H2 scissoring vibration of deoxyribose. The 1426cm-1 band is also contributed by C4-C9 stretching and C8-H deformation modes of adenine and guanine.(7)

In the IR spectrum of 1340-1200 cm-1 region encompasses weak NH vibrations and CH in-plane deformations of nucleic acids above 1278cm-1. The bands 1292 and 1278. The 1292cm-1 line has been assigned to a vibration of (C'-H) bending, and ribose ring vibrations of thymidine based on isotope edited studies of thymidine.

IR spectra of DNA in aqueous solution have 3 main absorption bands at 1053cm-1 (vibrations of the C-O-P sugar-phosphate backbone), 1090cm-1(symmetrical vibrations of phosphate groups) and 1230cm-1 (asymmetrical vibrations of phosphate groups. The positions of these bands are correspond to the IR spectrum of the native DNA in B-form in solution. The band at 1018cm-1(shoulder) can be associated with deoxyribose vibrations coupled to backbone vibrations, its strong hyperchromicity possibly reflecting the effect of the electronic polarization of the C-O-C deixyribose atoms close to guanine ring in the B structure.

IR bands in the 750-850 cm-1 spectral have been assigned to vibrations which involve a large contribution from oxygen-phosphorous stretching of the phosphodiester(O-P-O) linkages and oxygen-phosphorous stretching of the phophodioxy(PO2-) group. Because of obvious coupling between sugar and phosphate groups, these bands are extraordinarily sensitive to the nucleic acid backbone conformation and provide a firm empirical basis for DNA conformational analysis.

FT-IR spectrum of calf thymus DNA exhibits a moderate band at 886 originates from vibration associated with the 3',5'-phosphodiester network (C-O-P-O-C). The principle B-marker near 842cm-1, the present spectrum in H2O shows two bands near ~820-810 and at 800-780 cm-1 . The former(~820-810) occurs at the position expected for the A-DNA backbone marker and indicates a minor contribution from such a conformation to aqueous DNA structure while the latter (800-780) is expected for the Z-conformation.

The 779 cm-1 band in H2O solution is composite of two major bands, one due to the cytosine ring breathing mode, and the other due to phosphodiester symmetric stretching vibration.

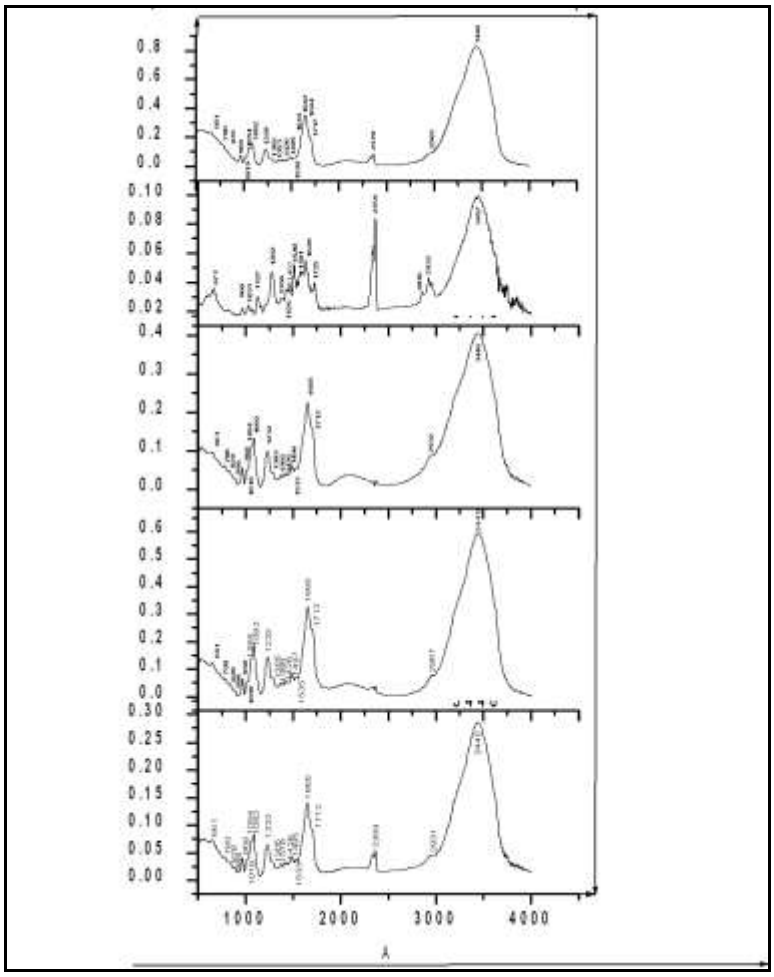
The 655(dT) cm-1 band expected in H2O solution has been assigned to an in-plane bending mode of thymine coupled with the deoxyribose moiety in which the six member ring undergoes a skeletal deformation while the C2=O and C4=O groups simultaneously bend in-phase with one another.

Curcumin complex-DNA Interaction

r=1:50 and 1:20

The infrared spectrum of curcumin-DNA complex has offered an evidence for the direct binding of curcumin to DNA. The vibrational frequencies of the NH and OH group of free DNA appeared around 3442cm-1 has shifted into 3446cm-1. The broadening in free DNA band is mainly attributed to the intramolecular H-bonding and the observed increase in NH band on curcumin interaction is indicative of the interaction of curcumin with DNA bases. The interaction of curcumin could also be envisaged from the observed change mainly in the vibrational frequency of OH of curcumin appeared around 3442 has changed into 3446 cm-1 in curcumin-DNA complex. Thus the vibrational frequencies NH band in DNA and OH in curcumin has changed in complex. Obviously representing the effective interaction of curcumin OH with NH of DNA bases which could be possible through H-bond interaction.

The infrared spectrum of Curcumin-DNA complex implies that the vibrational frequency of NH/OH of free DNA at 3550-3100 cm-1 and the vibrational frequency of OH of curcumin at 3438 has shifted towards 3452 in the complex. This suggests that the NH of free DNA bases and OH of curcumin are involved in mutual H-bonding interaction.



The absorption bands in the region 1800-1550 cm-1, due to the in-plane DNA vibrational frequencies.2,3,4,13,14) A major increase in the intensity was observed mainly for a guanine band at 1710 cm-1 with shifted towards a lower frequency at 1703 cm-1.(15) A similar trend was observed for the DNA band at 1710 cm-1, in plane stretching vibration of double bond of guanine (C7=N) located at the major groove. With

respect to both intensity increase and shifting of the band, the present result suggest that curcumin interacts mainly with the guanine-N7 reactive site located in the major groove of dsDNA. Similar spectral changes (intensity increase and downward shifting) were observed for the band at 1651 cm-1(T,G,C) mainly for thymine and 1610 cm-1(mainly adenine). The intensity decrease of these vibrations is also associated with the shift of the bands of 1651-1653 cm-1 and 1612 cm-1. The observed spectral changes are due to the curcumin with the A-T base pairs is weak since the overall spectral changes(intensity variations) of the A-T bands are not so large. A similar decrease in the intensity of several A-T IR lines was observed by Ahamad etal.,2003(16).

However the major intensity increase was observed in the infrared bands of 1661 cm-1 (mainly thymine) and 1610 cm-1 (mainly adenine). The intensity increase of these vibrations is also associated with the shift of the bands of 1661-1657 cm-1 and 1610-1604 cm-1. The observed spectral changes are due to the curcumin-DNA interaction thymine O-2 and adenine N-7 atoms (with O-H group of curcumin) that are not normally involved in Watson-Crick hydrogen bonding network. Such interaction does not bring about helix destabilization.

Other DNA vibrational frequencies in the region 1550-1250cm-1 showed minor spectral changes upon curcumin complexation. The band at 1492cm-1, which is related largely to the cytosine residues(ref), exhibited no major shifting (1cm-1), and its relative intensity did not change significantly in curcumin-DNA complexation. Thus, the possibility of an interaction between cytosine and the curcumin cannot be included. It is worth mentioning that the weak interaction at 1492 cm-1 in the spectrum of curcumin-DNA complexation. Therefore, our results support the existence of hydrophobic interactions between the curcumin cations and DNA duplex. This is indicative of less perturbation of cytosine bases in curcumin-DNA complex formation. Similar spectral changes were observed in the IR spectra of caffine-, theophylline-, and taxol-DNA complexes (Ahamad ouameur et al., 2004; Nafisi et al; 2008)(16)

To establish a possible interaction of curcumin with the backbone phosphate group, the infrared spectra of dsDNA in the region 1250-1000 cm-1 were examined. The two strong absorption bands located at 1225 and 1088 cm-1 are assigned mainly to the asymmetric and symmetric stretching vibrations of the PO2- groups, respectively(2,3,4,17). An increase in the relative intensity of the ν_{as}PO2- absorption band (1230 cm-1) no shift was observed in this vibration in curcumin-DNA complexation. The strongest absorption in the phosphate backbone region arises from the symmetric vibration of the O=P=O group. The maximum of this band is located at 1088 cm-1. It does not change position significantly upon interaction with curcumin. No changes of note occur for the deoxyribose absorption at 1018 cm-1(16).

DNA Conformation-The region 1000-700 cm-1 is of particular interest for DNA conformation. The band at 968 cm-1, assigned to the C-O and C-C stretching vibration of deoxyribose. This band observed at the same frequency, suggesting that no direct curcumin-sugar interaction occurred in this curcumin-DNA complexation. The peaks at 938, 895 and 836 cm-1, known as the B-DNA markers are stable with only minor intensity changes confirming that the DNA structure remains within the B-family when interacting with the curcumin.

DNA structural Conformation at Higher curcumin concentration(r>1/10)

The broad band at 3442 cm-1 in free DNA is mainly attributed to the intramolecular H-bronding and the observed reduction in this on drug interaction is indicative of the interaction of curcumin with DNA bases. The interaction of curcumin could also be envisaged from the observed change mainly in the vibrational frequency of OH of curcumin appeared around 3442 cm-1 has changed into3446 cm-1 in DNA-curcumin complex. Thus the vibrational frequencies of NH band in DNA and OH in drug has changed in complex, obviously representing the effective interaction of drug OH with NH of DNA bases which could be possible through H-bond interaction.(1)

The IR band at 2921 cm-1 in curcumin free DNA observed the methylene C-H stretching vibration shifted to 2929cm-1 with major decreased intensity. This result indicate the methylene C-H stretching band was followed upon formation of complexes with curcumin.(16)

The region 1800-1550 cm-1 called “in-plane DNA vibrational region” contains bands due to stretching vibrations of double bonds including carbonyl groups and inplane deformation of N-H groups of G, C, A and T.

The 1710 cm-1 band of curcumin free DNA due to in plane stretching vibration of double bond of guanine (C7=N) located at the major groove shifts to lower frequency near 1704 cm-1 in curcumin-DNA complex and decrease in intensity. This result indicates curcumin binding at N7 atom of guanine . Ahmad et al. have made similar observations in IR spectra of DNA with mg(II) and Ca(II) in aqueous solutions.(15)

The band at 1651 cm-1 in curcumin free DNA, due to T, G, C mainly for T. The complex formation stimulates the upshift of the 1651 thymine band to the final position at 1658cm-1. This band corroponds to the C5C4-C4O vibrations of thymine groups with 37% contribution of the C4O group. With respect to both intensity decrease and shifting to lower frequency, the curcumin molecule in this position could change the DNA structure via deformation of the hydrogen bond between NH2 group of adenine and the C4O group of thymine molecule. This structure deformation leads to a change of the thymine band position in the IR spectrum of the complex.

An absorption at 1643 cm-1 , which is due either to an in-plane ring vibration of cytosine or C4=O4 of thymine or both, which is exhibited higher frequency shift with major decreased intensity. The loss of intensity has been attributed to DNA aggregation, condensation or helix stabilization.

The localization of the curcumin molecule in the major groove of DNA can influence the NH2 deformation vibration of the adenine molecule. This is the reason why the complex formation leads to the appearance of the 1610 cm-1 band (fig) which is attributed to the adenine δNH2-C5C6-C6N6' vibrations with 73% of the δNH2 group vibrations contribution (12).

Other DNA vibrational frequencies in the region 1550-1250 cm-1 showed major spectral changes upon curcumin complexation. The DNA IR band near 1492 cm-1 is due primarily to a vibration localized in the cytosine ring.

It is important to note that the cytosine band at 1530 cm-1 lost intensity and shifted toward a lower frequency at 1527 cm-1, whereas the band at 1492 cm-1 related to the cytosine and guanine modes lost intensity and shifted toward a higher frequency at 1494 cm-1. The observed spectral changes can be attributed to the protonation of cytosine N3 and cation chelation through the N7 and O6 group of guanine bases at this concentration.

To establish a possible interaction of curcumin with the backbone phosphate group, the infrared spectra of dsDNA in the region 1250-1000 cm-1 were examined. The two strong absorption bands located at 1230 and 1088 cm-1 are assigned mainly to the asymmetric and symmetric stretching vibrations of the PO2- groups, respectively. The decrease in the relative intensity of the vasPO2- absorption band (1231 cm-1) was observed with the shift of this vibration 1229 and the symmetric band in curcumin-DNA complexes (r=1:10 and r=1:5).

The parent IR bands are due to vibrations localized in phosphate group of the DNA backbone (C3'-O-P-O-C5' phosphodiester stretch at 779cm-1 and PO2- phosphodioxy stretch at 1094 cm-1), DNA phosphates are indicated as the primary sites of curcumin binding. This binding represents direct experimental evidence in favor of non-specific binding of curcumin to CT-DNA of differing in different ratios; it is consistent with models proposing electrostatic attractions between the DNA phosphates and curcumin cationic groups as the driving force for curcumin-induced DNA condensation at higher concentrations of curcumin.

Thus, from the IR data it is clear that curcumin interacts with N7 positions of adenine and guanine and that this interaction leads a partial deformation of the Hydrogen bonds between adenine and thymine bases. An interesting question is which part of the curcumin molecule is implicated in this interaction? With regards to the curcumin structure and the nature of the IR spectra any type of interaction of curcumin to the DNA structure can be excluded. As the N& positions of guanine and adenine are proton-acceptor sites of the purine rings, it seems very probable that curcumin interacts with guanine and adenine via a hydrogen bond formation between the protons of its amino group and the N& positions of the purines. An effect of hydrogen bonding on the adenine ring vibrations, in solvents with varied hydrogen bonding properties, was recently presented by Fujimoto et al. In this study the upshift of the denine band has been interpreted as being caused by a strong hydrogen bond formation with the adenine proton accepter sites (N1, N3 and N7). The N7C5 group contribution represents in this vibration and N1 nitrogen forms a hydrogen bond.

UV-Vis studies

Introduction

Absorbance in the UV/Vis region has been successfully used for the analysis of curcumin-DNA interactions. In this study, the curcumin were incubated in the presence and absence of various concentrations of DNA. The changes in their absorbance at 426nm were measured with respect to the DNA concentration.

A more detailed investigation of their interaction was made by UV spectroscopy. Fig..shows the UV spectra of the curcumin-DNA system. It was found that the maximum absorptions of free curcumin and DNA were located at 426 and 260nm respectively. The absorbance of curcumin at 426nm increased and ha a slight blue shift with increasing the concentration of DNA. In order to make sure that the absorbance of the mixture system was different from the sum values of each component, detailed calculation of the absorbance of free curcumin and added DNA demonstrated that a binding event occurred between them, since $A_{curcumin+DNA}$ is larger than $A_{curcumin}$. This means that there was a hypochromic effect in the curcumin-DNA system. The characteristic absorption band of DNA was found to show up when DNA was added above a desired concentration, which revealed the emergence of excess nucleic acid in the system. The increase in the absorbance of curcumin with the presence of DNA at a low concentration could be attributed to a weak interaction between DNA and curcumin, forming a curcumin-DNA complex. Due to the different structure properties of curcumin and DNA molecules, the information on the interaction mode of curcumin with DNA could be judged either by the properties of DNA or by curcumin. The characteristic hypochromism, hypsochromism, sharp isosbestic points and and saturation at high DNA concentrations suggest a simple two state transition between the free and bound ligand in curcumin-DNA complexation. The absolute changes in intensity and the degree of blu shifting are slightly different for each concentration as described above, but the overall spectral changes for all reported titrations suggested simple two state equlibria and essentially indicate strong intermolecular interaction involving effective overlap of pai-electron cloud of these molecules with the DNA bases. The results of absorption spectral titration in case of curcumin-DNA complexation were converted to Scatchard plots. The binding parameters of curcumin to the DNA are presented to the table.

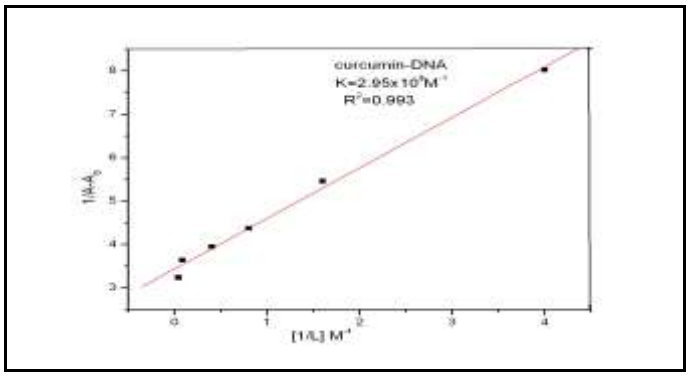


Fig.2

In the double reciprocal plot of $1/(A-A_0)$ Vs $1/L_0$ [figure 2], the binding constant(K) is given by ratio of slope to intercept. The K value ($2.95x10^5M^{-1}$) is lower than those observer for typical classical intercalators (ethidium – DNA, $7x10^7M^{-1}$ in 40mM Tris-HCl buffer, pH 7.9, and $1.4x10^6M^{-1}$ in 40mM NaCl-20mM Tris-HCl with a proven DNA-binding mode involving the complete insertion of the planar molecules between the base pairs. Similar binding constant was reported for DNA complexes with A_{zT} taxol, chlorophyll, chlorophyllin and biogeneic polyamines. This is indicative of binding of the curcumin with DNA host with an affinity less than the classical intercalators.

Conclusion:

The interactions of curcumin with ct-DNA were studied using UV-Vis, and FT-IR spectroscopy. UV-Vis experiments reveal binding constants of 2.95 $x10^5$ M^{-1}.Small molecules interacting with double standard DNA (dsDNA) can be classified according to their binding modes: intercalators bind in the minor or major

groove of DNA. Fluorescence and UV-Vis data revealed that the interaction of the curcumin with DNA is not a classical intercalator.

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