DNA−Chlorophyllin Interaction

Jean-F. Neault and H. A. Tajmir-Riahi*

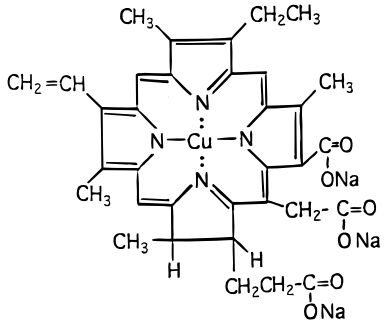
Department of Chemistry−Biology, University of Québec at Trois-Rivières, C.P. 500, TR (Québec), Canada G9A 5H7†

Received: June 25, 1997; In Final Form: October 16, 1997

Porphyrins and their metal derivatives are strong DNA binders with association constants of around 10^6 M^{-1}. Some of these compounds have been used for radiation sensitization therapy of cancer and are targeted to interact with cellular DNA. Chlorophyllin (CHLN), a food-grade derivative of chlorophyll (CHL), the ubiquitous green plant pigment widely consumed by humans, is a potent inhibitor of experimental carcinogenesis. The aim of this study was to examine the interaction of calf-thymus DNA with chlorophyllin in aqueous solution at physiological pH, with CHLN/DNA(phosphate) molar ratios (r) 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 CHLN binding mode, binding constant, sequence selectivity, DNA secondary structure, and structural variations of the DNA−CHLN complexes in aqueous solution. Spectroscopic evidence showed that at low pigment concentration (r = 1/80), CHLN binds DNA via intercalative mode into the G−C and A−T-rich regions with a minor interaction toward the backbone PO_2 group (outside binding). At r > 1/80, a partial reduction of B-DNA structure in favor of A-DNA occurs upon drug complexation. At high drug content (r > 1/20), a minor helix opening is observed. The calculated binding constant $K = 3.56 \times 10^3$ M^{-1} shows chlorophyllin as a weak DNA intercalator. The DNA−chlorophyllin complexation is rather different from that of the DNA−chlorophyll interaction, in which chlorophyll binding is mainly through the backbone PO_2 group with minor cation−base interaction (groove binding).

Introduction

Diet has been shown to play an important role in human cancer risk.[1−4] Although much attention has been focused on dietary carcinogens and promoters, recent evidence shows that protective factors within the diet are also important in cancer risk. Several chemical components of fruits and vegetables have been isolated and purified and shown to be effective against carcinogenesis in experimental animal models.[5−8] Chlorophyll and its derivatives have been shown to exert profound antimutagenic behavior against a wide range of potential human carcinogens.[7−9] Similarly, chlorophyllin (structure **1**), a food-grade derivative of chlorophyll, has been used historically in the treatment of several human conditions, with no evidence of human toxicity,[10−12] and recently it was used as a potent inhibitor of aflatoxin B_1 hepatocarcinogenesis in rainbow trout.[13]



CHLOROPHYLLIN

1

It has been suggested that the antimutagenic activity of chlorophyllin comes from its strong complexation with parent mutagens or their intermediates, scavenging of free radicals and active oxygen species, and suppression or interference with metabolic activation by specific cytochrome (P-450) and other metabolizing enzymes.[14−17] Chlorophyllin was also shown in vivo to efficiently inhibit precarcinogenic target organ DNA adduction by aflatoxin B_1 in trout[16] and 2-amino-3-methylimidazol [4,5-f]quinoline in rat.[18] Since the major target of these anticancer drugs can involve DNA or DNA adducts, the interaction of chlorophyllin with DNA has major biological importance, and thus, the present study is designed to investigate DNA−CHLN complexation in vitro and to provide structural information regarding the drug binding mode, sequence preference, and DNA secondary structure, using infrared spectroscopy. Our structural information provides, to our knowledge, the first spectroscopic evidence regarding DNA−CHLN interaction and should help to elucidate the nature of this biologically important complex formation.

Recently, we have reported[19] the interaction of chlorophyll *a* with calf-thymus DNA, in which CHL binding was mainly through the backbone PO_2 group (external binding) via chlorophyll Mg cation with minor interaction with the guanine and adenine N-7 atoms (groove binding). The CHL interaction resulted in a minor helix opening and a partial reduction of B-DNA structure in favor of A-DNA.[19] Vibrational spectroscopy has been widely used to analyze the nature of DNA−drug complexation and to provide structural information at a molecular level.[20] Recently, we used vibrational spectroscopy (infrared and Raman) for the structural characterization of

† Abbreviations: CHLN, chlorophyllin; CHL, chlorophyll; DES, diethylstilbestrol; H_2TMPyP4, tetrakis(4-*N*-methylpyridyl)porphyrin; FTIR, Fourier transform infrared; A, adenine; C, cytosine; G, guanine; T, thymine; r, pigment/DNA(P) molar ratio.

* Corresponding author: Tel, (819) 376-5052 (ext. 3321); Fax, (819) 376-5084; E-mail, Tajmirri@uqtr.uquebec.ca.

S1089-5647(97)02082-8 CCC: $15.00
Published on Web 02/11/1998

DNA−DES (DES = diethylstilbestrol), DNA−aspirin, DNA−vitamin C, and DNA−carbohydrate[21−24] as well as DNA−cation[25,26] and protein complexes.[27] We believe that FTIR difference spectroscopy can be also applied here, to characterize the nature of the DNA−pigment interaction and to provide structural information on the DNA−CHLN complexes formed in aqueous solution.

In this work, FTIR difference spectroscopy is applied to study the interaction between calf-thymus DNA and chlorophyllin in aqueous solution at physiological pH, with CHLN/DNA(P) molar ratios of 1/80 to 1/2. Spectroscopic evidence regarding the pigment binding mode, binding constant, sequence selectivity, and biopolymer secondary structure is provided. Furthermore, comparisons were made between the DNA−chlorophyllin and DNA−chlorophyll complexes and those of the corresponding metalloporphyrin−DNA adducts, and the results are reported here.

Materials and Methods

Materials. The crystalline chlorophyllin (copper-trisodium salt) was from Sigma Chemical Co. and used as supplied. Highly polymerized type I calf-thymus DNA sodium salt (7% Na content) was purchased from Sigma Chemical Co. and deproteinated by the addition of $CHCl_3$ and isoamyl alcohol in NaCl solution. Other chemicals were of reagent grade and used without further purification.

Preparation of Stock Solutions. Sodium−DNA was dissolved to 2% w/w (0.05 M DNA(phosphate)) in 0.05 M NaCl and 1 mM sodium cacodylate (pH 7.30) at 5 °C for 24 h with occasional stirring to ensure the formation of a homogeneous solution. The appropriate amount of chlorophyllin (0.3−12.5 mM) was prepared in distilled water and added dropwise to DNA solution to attain desired CHLN/DNA(P) molar ratios of 1/80, 1/40, 1/20, 1/10, 1/4, and 1/2 at a final DNA concentration of 1% w/w or 0.025 M DNA(phosphate). The solution pH was adjusted to 7.30−6.80, using NaOH solution. The infrared spectra were recorded 3 h after mixing of CHLN and DNA solutions. The infrared spectra of DNA-CHLN complexes with $r > 1/2$ could not be recorded in solution due to solid gel formation.

Physical Measurements. The infrared spectra were recorded on a Bomem DA3-0.02 FTIR spectrometer equipped with a nitrogen-cooled HgCdTe detector and KBr beam splitter. The solution spectra were taken using AgBr windows with resolution of 2−4 cm^{-1} and 100−500 scans. The sample preparation and spectral measurements were carried out under green light (to avoid photodegradation of chlorophyllin in CHLN−DNA complexes). The water subtraction was carried out as in our previous report.[21,22] A good water subtraction is achieved as shown by a flat baseline around 2200 cm^{-1}, where the water combinatiom mode[28] is located (this portion of spectra is not shown). The difference spectra [(DNA solution + CHLN solution) − (DNA solution)] were produced, using a sharp DNA band at 968 cm^{-1} as internal reference. This band, due to deoxyribose C−C stretching vibrations, exhibits no spectral changes (shifting or intensity variations) on CHLN−DNA complexation, and it was canceled upon spectral subtraction. The intensity ratios of several DNA in-plane vibrations, related to A−T and G−C base pairs and the PO_2 stretching modes, were measured (with respect to the reference band at 968 cm^{-1}), as a function of CHLN concentration with an error of ±2%. These intensity ratio measurements are used to quantify the amounts of CHLN bindings to the backbone PO_2 group and DNA bases. The detailed infrared spectral manipulations and intensity ratio calculations are presented in our recent publications.[21−26]

Results and Discussion

DNA−CHLN Complexes. At low chlorophyllin concentration ($r = 1/80$), the CHLN binds via intercalative mode into the G−C and A−T regions. Evidence for this comes from the minor increase in the intensity (5%) of the mainly G-band at 1717 cm^{-1} and T-band at 1663 cm^{-1} [28−33] (Figure 2). These intensity changes were associated also with the shift of the bands at 1717 (mainly G) to 1715 and 1663 (mainly T) to 1666 cm^{-1}. Similarly, the mainly adenine band at 1609 cm^{-1} shifted toward a lower frequency at 1605 cm^{-1} (spectrum is not shown). A minor intensity increase (5%) was also observed for the backbone PO_2 antisymmetric stretching vibration at 1222 cm^{-1} upon CHLN complexation (Figure 2). The spectral changes observed for the phosphate band at 1222 cm^{-1} are also due to some degree of Cu−PO_2 interaction (outside binding). The positive derivative features observed at 1710, 1660, and 1214 cm^{-1}, in the difference spectra of CHLN−DNA complexes, are due to the increase in the intensity of the bands at 1717, 1663, and 1222 cm^{-1} (Figure 1, $r = 1/80$).

The calculation of the binding constants was carried out as reported for other pigment complexes with DNA, RNA, and mononucleotides.[34−38] By assuming that there is only one type of interaction (intercalation) between CHLN and DNA molecule, the following equations can be established.

$$\text{DNA} + \text{CHLN} \Leftrightarrow \text{DNA:CHLN} \qquad K \tag{1}$$

$$K = \frac{[\text{DNA:CHLN}]}{[\text{DNA}][\text{CHLN}]} \tag{2}$$

Since chlorophyllin intercalation is limited to the G−C and A−T bases, the relative intensity variations of the bands at 1717 cm^{-1} (mainly G) and at 1663 cm^{-1} (mainly T) were calculated at each drug concentration. The calculated intensities were used as a function of pigment concentration to estimate the K(G) for guanine and K(T) for thymine bases. The double reciprocal plot of $1/(I - I_0)$ vs 1/[CHLN] is linear, and the binding constant (K) can be estimated from the ratio of the intercept to the slope. I_0 is the initial relative intensity of the specific DNA absorption band, and I is the relative intensity at different CHLN concentrations. The overall binding constant (K) for CHLN−DNA complexes is estimated to be 3.56×10^3 M^{-1}. Similar methods, based on the intensity ratio variations of the Raman and infrared vibrational frequences, have been used to determine the binding constants of diethylstilbestrol (intercalating drug)[38] and chlorophyll[19] to polynucleotides and the CH_3Hg^+ cation to mononucleotides.[37]

The value of $K = 3.56 \times 10^3$ M^{-1} estimated for the DNA−CHLN complexes is consistent with the chlorophyllin being a weak DNA intercalator. The association constants of the CHLN−DNA complexes are smaller than those of the strong DNA intercalators, such as ethidium bromide, acridine orange, methylene blue, and other pigments with binding constants ranging from 10^5 to 10^6 M^{-1}.[39−50]

The drug distributions around A−T and G−C base pairs (based on the binding constant $K = 3.5 \times 10^3$ M^{-1} and pigment concentration 6.5×10^{-4} M) were 20% with the A−T bases and 15% around the G−C, and less than 10% of CHLN were PO_2 bound (Figure 3).

It is also important to note that a recent X-ray crystallographic study showed that the CuTMPyP4 [copper(II)-*meso*-tetra(*N*-methyl-4-pyridyl)porphyrin] is hemiintercalated into the G−C-

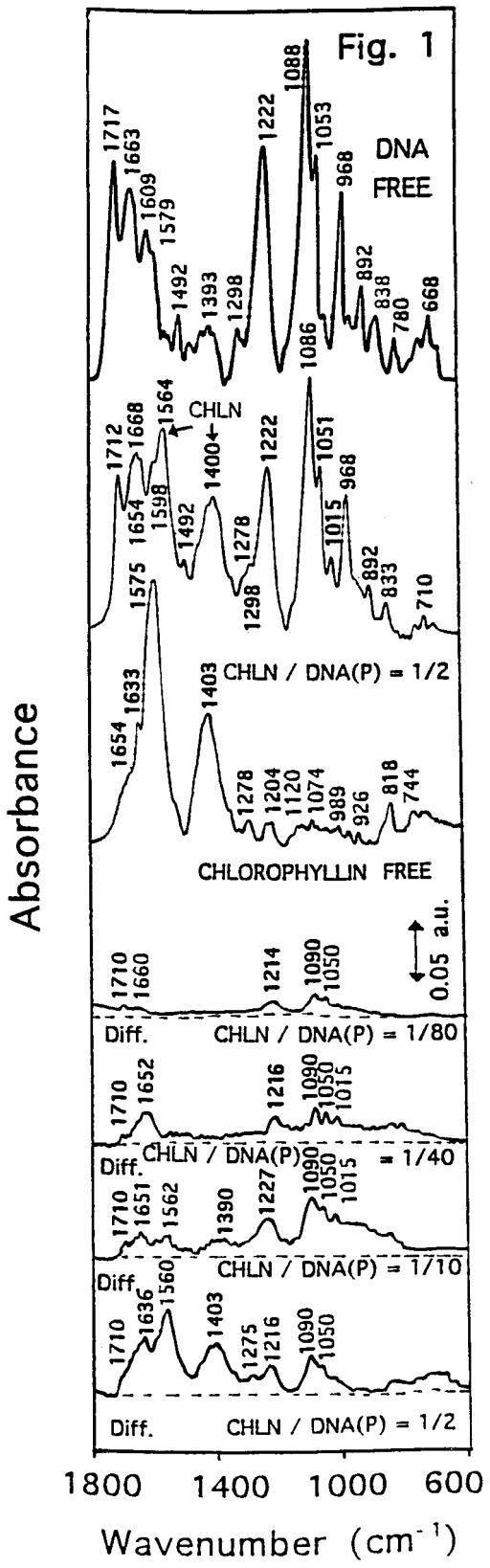


Figure 1. FTIR spectra (top three curves) and difference spectra [(DNA solution + CHLN solution) − (DNA solution)] (bottom four curves) of the uncomplexed calf- thymus DNA and its chlorophyllin (CHLN) adducts in aqueous solution at pH = 6.80−7.30 with different CHLN/DNA(P) molar ratios in the region 1800−600 cm^{-1} (the baselines are shown as broken lines).

rich region of a hexamer duplex $[d(CGATCG)]_2$ with a major electrostatic interaction between positively charged nitrogen atoms of the pyridyl rings and negatively charged backbone

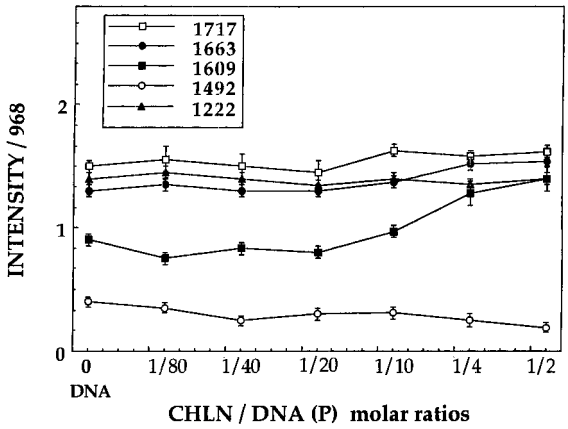


Figure 2. Intensity ratio variations for several DNA in-plane vibrations at 1717 (G, T), 1663 (T, G, A, C), 1609 (A), 1492 (C, G), and 1222 cm^{-1} (PO_2 stretch) as a function of chlorophyllin (CHLN) concentration (different CHLN/DNA(P) molar ratios).

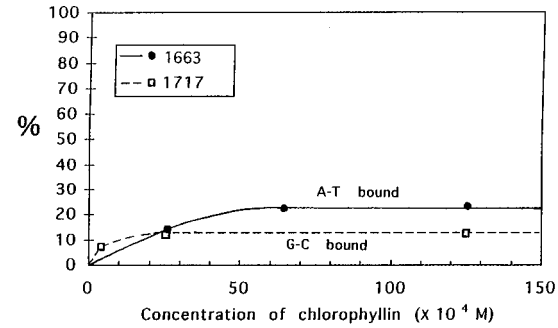


Figure 3. Calculated distributions of the CHLN bound to the A−T (1663 cm^{-1}, mainly thymine) and to the G−C (1717 cm^{-1}, mainly guanine) regions in aqueous solution with DNA concentration of 0.025 M (phosphate) as a function of CHLN concentration (M) based on $K = 3.56 \times 10^3\ M^{-1}$ at a pigment concentration of 6.5×10^{-3} M.

phosphate oxygen atom.[50] However, the chlorophyllin structure is rather different from that of the copper−porphyrin complex intercalated into the G−C region. Similarly, the calf-thymus DNA with major A−T base pairs (60%) and less G−C content (40%) is different from that of the hexamer $[d(CGATCG)]_2$ oligonucleotide. The observed structural dissimilarities between copper porphyrin and CHLN on one hand and the oligonucleotide hexamer with calf-thymus DNA on the other hand are the main reasons for the different binding modes of the CHLN and copper−porphyrin in these DNA complexes.

At $1/80 < r < 1/20$, a minor decrease in the intensity (5−10%) of the bands at 1717, 1663, and 1222 cm^{-1} was observed upon drug interaction (Figure 2). The observed spectral changes were associated with the loss of the intensity and shifting of the B-DNA indicator[33] at 838−833 cm^{-1} (deoxyribose−phosphate mode) in the spectra of the CHLN−DNA complexes. The shift of the B-DNA indicator from 838 to 833 cm^{-1} was accompanied by the shift of the other B-DNA marker band at 1717−1712 cm^{-1}.[33] The observed spectral changes are related to a partial reduction of B-DNA structure toward A-DNA. Similar behaviors were observed in the infrared spectra of the chlorophyll−DNA complexes, where a partial reduction of the B-DNA structure occurred in favor of the A-DNA conformation.[19]

At $r > 1/20$, a minor helix destabilization occurred upon drug complexation. Evidence for this comes from an increase in the intensity (10−15%) of the bands at 1717, 1663, 1609, and 1222 cm^{-1} (Figure 1, $r = 1/10$). The presence of several positive derivative features at 1710, 1651, and 1227 cm^{-1} in the

difference spectra of CHLN−DNA complexes are also consistent with the intensity increase of DNA in-plane vibrations (Figure 1, $r = 1/10$). The intercalation of the porphyrin and copper−porphyrin CuTMPyP4 complexes also resulted in a minor helix destabilization of DNA oligonucleotide.[41,50] Similarly, the melting of DNA double helix by thermal denaturation or by cation coordination also resulted in a major increase in the intensity of several DNA vibrational frequencies.[20,25,26]

At high pigment concentrations ($r = 1/4$ and 1/2), the major intensity variations observed for the A−T vibrations were combined with considerable shiftings of the bands at 1663 (T) to 1668 cm^{-1} and 1609 (A) to 1598 cm^{-1} upon drug intercalation (Figures 1 and 2, $r = 1/4$ and 1/2). The observed spectral changes are indicative of further drug interaction with the A−T-rich regions. It is important to note that at high pigment concentration ($r = 1/2$) most of the DNA in-plane vibrations in the region of 1700−1500 cm^{-1} are overlapped by the strong CHLN vibrational frequencies (Figure 1, difference spectrum for 1/2).

Additional evidence for CHLN−DNA complexation also comes from the major spectral changes of the chlorophyllin vibrational frequencies upon DNA interaction. A strong vibration at 1575 cm^{-1} in the spectrum of the free pigment, related to the porphyrin ring C═C and C═N stretching modes,[44] shifted toward a lower frequency at 1564 cm^{-1} upon drug interaction (Figure 1). Similarly, the band at 1403 cm^{-1} due to the ring C−N and C−O stretching vibrations[44] appeared at 1400 cm^{-1} in the spectra of CHLN−DNA complexes (Figure 1). The observed spectral shiftings are due to major structural changes of the porphyrin rings upon DNA interaction. It should be noted that the strong positive derivative features observed at 1636, 1560, and 1403 cm^{-1} in the difference spectra of CHLN−DNA complexes formed at high drug content come from the chlorophyllin vibrations and they are not related to DNA molecule (Figure 1, $r = 1/2$).

The overall spectral changes observed are due to a major drug intercalation via A−T and G−C base pairs and stabilization of these complexes by some degree of drug−PO_2 interaction. Similar spectral changes were observed in the Raman spectra of other copper−porphyrin complexes intercalated into the G−C and A−T-rich regions of the native and synthetic DNAs.[39−40]

Comparisons between CHLN−DNA and CHL−DNA Complexes

Porphyrins and their metal derivatives intercalate in both native and synthetic DNAs.[41−50] Porphyrins also bind DNA via outside (nonintercalative mode) binding.[42] The mode of binding is controlled by porphyrin shape and charge. Porphyrins with axial ligands at fifth and sixth coordination sites of cation sterically block intercalation. Therefore, free porphyrin H_2-TMPyP4 and four-coordinate metal derivatives such as NiTPMyP4, CuTPMyP4, and PdTMPyP4 intercalate, whereas five- or six-coordinate MnTMPyP4, FeTMPyP4, CoTMPyP4, and ZnTMPyP4 bind via nonintercalative modes.[45,46]

Chlorophyllin with four-coordinate copper cation in the center is a good candidate for DNA intercalation, while chlorophyll with five- or six-coordinate magnesium ion (in aqueous solution) binds DNA via nonintercalative mode.[19] On the basis of the infrared spectroscopic results presented here for the first time, it is clearly evident that chlorophyllin binds calf-thymus DNA via intercalative mode into the G−C and A−T-rich regions with minor pigment−PO_2 interaction (outside binding). At high pigment content, minor helix destabilization occurs with a partial reduction of the B-DNA structure in favor of A-DNA. On the other hand, chlorophyll binding with calf-thymus DNA was mainly through Mg−PO_2 interaction (outside binding) with minor Mg−N-7 coordination (groove binding).[19] Chlorophyll complexation resulted also in a major reduction of B-DNA structure in favor of A-DNA with a partial helix opening at high pigment content.[19] The calculated binding constants are $K = 1.13 \times 10^4\ M^{-1}$ for CHL−DNA and $3.56 \times 10^3\ M^{-1}$ for CHLN−DNA adducts. The differences in the stability of these pigment−DNA complexes can be attributed to a stronger interaction of Mg−PO_2 (external binding) in the CHL−DNA and a weak intercalative mode of the chlorophyllin in the CHLN−DNA complexes. The larger size of the chlorophyllin (with respect to chlorophyll) plays an important role in the formation of less stable metalloporphyrin−DNA complexes.

Acknowledgment. This work is supported by a grant from the Natural Sciences and Engineering Research Council of Canada (NSERC).

References and Notes

(1) Doll, R. *Proc. Nutr. Soc.* **1990**, *49*, 119−131.
(2) Doll, R.; Peto, R. J. *Natl. Cancer Inst.* **1981**, *66*, 1191−1308.
(3) Farber, E. *Am. J. Pathol.* **1982**, *106*, 271−296.
(4) Wattenberg, L. W. *Proc. Am. Assoc. Cancer Res.* **1990**, *32*, 461−463.
(5) Hayatus, H.; Arimoto, S.; Negishi, T. *Mutat. Res.* **1988**, *202*, 429−446.
(6) Dragsted, L. O.; Strube, M.; Larsen, J. C. *Pharmcol. Toxicol.* **1993**, *72*, 116−135.
(7) Lai, C.; Butler, M. A.; Matney, T. S. *Mutat. Res.* **1980**, *77*, 245−250.
(8) Kimm, S.; Tchai, B.; Park, S.; Kang, S. *Korean J. Biochem.* **1982**, *14*, 1−7.
(9) Kimm, S.; Park, S. *Korean J. Biochem.* **1982**, *14*, 47−59.
(10) Ong, T.; Whong, W.; Stewart, J.; Brockman, H. E. *Mutat. Res.* **1986**, *173*, 11−115.
(11) Harrison, J. W. E.; Levin, S. E.; Trabin, B. *J. Am. Pharm. Assoc.* **1954**, *43*, 722−737.
(12) Young, R. W.; Beregi, J. S. *J. Am. Geriat. Soc.* **1980**, *28*, 48−50.
(13) Breinholt, V.; Hendricks, J.; Pereira, C.; Arbogast, D.; Bailey, G. *Cancer Res.* **1995**, *55*, 57−62.
(14) Newmark, H. L. A. *Nutr. Cancer* **1984**, *6*, 58−70.
(15) Arimoto, S.; Negishi, T.; Hayatsu, H. *Cancer Lett.* **1980**, *11*, 29−33.
(16) Dashwood, R. H.; Breinholt, V.; Bailey, G. *Carcinogenesis (London)* **1991**, *12*, 939−942.
(17) Romert, L.; Curvall. M.; Jenssen, D. *Mutagenesis* **1992**, *7*, 349−355.
(18) Dashwood, R. H. *Carcinogenesis (London)* **1992**, *13*, 113−118.
(19) Neault, J. F.; Tajmir-Riahi, H. A. Submitted for publication in *J. Phys. Chem.*
(20) Spiro, T. G. *Biological Applications of Raman Spectroscopy*; Wiley: New York, 1987; pp 81−133.
(21) Neault, J. F.; Tajmir-Riahi, H. A. *J. Biol. Chem.* **1996**, *271*, 8140−8143.
(22) Neault, J. F.; Naoui, M.; Manfait, M.; Tajmir-Riahi, H. A. *FEBS Lett.* **1996**, *382*, 26−30.
(23) Neault, J. F.; Naoui, M.; Tajmir-Riahi, H. A. *J. Biomol. Struct. Dyn.* **1995**, *13*, 387−397.
(24) Tajmir-Riahi, H. A.; Naoui, M.; Diamantoglou, S. *J. Biomol. Struct. Dyn.* **1994**, *12*, 217−234.
(25) Tajmir-Riahi, H. A.; Neault, J. F.; Naoui, M. *FEBS Lett.* **1995**, *370*, 105−108.
(26) Tajmir-Riahi, H. A.; Ahmad, R.; Naoui, M.; Diamantoglou, S. *Biopolymers* **1995**, *35*, 493−501.
(27) Ahmed, A.; Tajmir-Riahi, H. A.; Carpentier, R. *FEBS Lett.* **1995**, *363*, 65−68.
(28) Alex, S.; Dupuis, P. *Inorg. Chim. Acta* **1989**, *157*, 271−281.
(29) Keller, P. B.; Hartman, K. A. *Nucleic Acids Res.* **1986**, *14*, 8167−8182.
(30) Tsuboi, M. *Appl. Spectrosc. Rev.* **1969**, *3*, 45−90.
(31) Loprete, D. M.; Hartman, K. A. *Biochemistry* **1993**, *32*, 4077−4082.
(32) Starikov, E. B.; Semenov, M. A.; Maleev, V. Ya; Gasan, A. I. *Biopolymers* **1991**, *31*, 255−273.
(33) Brahms, S.; Brahms, J.; Pilet, J. *Isr. J. Chem.* **1974**, *12*, 153−163.
(34) Muller, W.; Crothers, D. M. *Eur. J. Biochem.* **1975**, *54*, 267−277.

(35) Tuite, E.; Kelly, J. M. *Biopolymers* **1995**, *35*, 419−433.
(36) Tuite, E.; Norden, B. *J. Am. Chem. Soc.* **1994**, *116*, 7548−7556.
(37) Tajmir-Riahi, H. A.; Langlais, M.; Savoie, R. *Biochim. Biophys. Acta* **1988**, *956*, 211−216.
(38) Neault, J. F.; Tajmir-Riahi, H. A. *J. Biol. Chem.* **1997**, *272*, 8901−8904.
(39) Wheeler, G. V.; Chinsky, L.; Miskovsky, P.; Turpin, P. Y. *J. Biomol. Struct. Dyn.* **1995**, *13*, 399−412.
(40) Kruglik, S. G.; Galievsky, V. A.; Chirvony, V. S.; Apanasevich, P. A.; Ermolenkov, V. V.; Orlovich, V. A.; Chinsky, L.; Turpin, P. Y. *J. Phys. Chem.* **1995**, *99*, 5732−5741.
(41) Fiel, R. J.; Howard, J. C.; Mark, E. H.; Datta Gupta, N. *Nucleic Acids Res.* **1979**, *6*, 3093−3118.
(42) Carvlin, M. J.; Fiel, R. J. *Nucleic Acids Res.* **1983**, *11*, 6121−6139.
(43) Sari, M. A.; Battioni, J. P.; Dupre, D.; Mansuy, D.; Le Pecq, J. B. *Biochemistry* **1990**, *29*, 4205−4215.
(44) Strahan, G. D.; Lu, D.; Tsuboi, M.; Nakamoto, K. *J. Phys. Chem.* **1992**, *96*, 6450−6456.
(45) Pasternak, R. F.; Gibbs, E. J.; Villafranca, J. J. *Biochemistry* **1983**, *22*, 2406−2414.
(46) Marzilli, L. G.; Banville, D. L.; Zon, G.; Wilson, W. D. *J. Am. Chem. Soc.* **1986**, *108*, 4188−4192.
(47) Kelly, J. M.; Murphy, M. J.; McConnell, D. J.; Oh Uigin, C. *Nucleic Acids Res.* **1985**, *13*, 167−184.
(48) Hudson, P. B.; Sou, J.; Berger, D. J.; McMillin, D. R. *J. Am. Chem. Soc.* **1992**, *114*, 8997−9002.
(49) Brun, A. M.; Harriman, A. *J. Am. Chem. Soc.* **1994**, *116*, 10383−10393.
(50) Lipscomb, L. A.; Zhou, F. X.; Presnell, S. R.; Woo, R. J.; Peek, M. F.; Plaskon, R. R.; Williams, L. D. *Biochemistry* **1996**, *35*, 2818−2823.