FEBS Letters 382 (1996) 26-30 FEBS 16707

Aspirin-DNA interaction studied by FTIR and laser Raman difference spectroscopy

J.F. Neault[a], M. Naoui[a], M. Manfait[b], H.A. Tajmir-Riahi[a,*]

[a]Department of Chemistry an. Biology, University of Québec at Trois-Rivières, C.P. 500, Trois-Rivières (Québec), G9A 5H7, Canada
[b]Laboratoire de Spectroscopie Biomoleculaire, UFR de Pharmacie, Université de Reims, 51096 Reims Cedex, France

Received 29 December 1995

Abstract The interaction of calf-thymus DNA with aspirin is investigated in aqueous solution at pH 7–6 with drug/DNA(phosphate) molar ratios of r = 1/40, 1/20, 1/10, 1/5, 1/2, 1 and 2. Fourier transform infrared (FTIR) and laser Raman difference spectroscopy are used to determine drug binding sites, sequence preference and DNA secondary structure, as well as the structural variations of aspirin-DNA complexes in aqueous solution. Spectroscopic evidence showed that at low aspirin concentration (r = 1/40), drug-DNA interaction is mainly through the backbone PO_2 groups and the A-T base pairs. Such interaction largely perturbs the phosphate vibration at 1222 cm^{-1} and the A-T bands at 1663 and 1609 cm^{-1} with no major helix destabilization. At higher drug concentration (r > 1/20), the participation of the G-C bases in drug-DNA complexation was evident by strong perturbations of the guanine and cytosine vibrations at 1717 and 1494 cm^{-1}, with a partial helix destabilization. A major alteration of the B-DNA structure towards A-DNA occurs on drug complexation. The aspirin interaction was through anion CO and $COOCH_3$ donor atoms with those of the backbone PO_2 group and DNA bases donor sites (directly or indirectly via H_2O molecules).

Key words: DNA; Drug; Aspirin; Conformation; Solution structure; FTIR; Raman spectroscopy

1. Introduction

Aspirin is an old drug, which falls into the category of the non-steroidal anti-inflammatory drugs (NSAID) and it is used extensively as a painkiller. In recent years, aspirin was found to reduce the risk of heart attack and stroke. Aspirin is also suggested to be effective against colorectal cancer, while it has no effect on other types of cancers such as lung, breast, ovary, testis, lymphoma and leukemia [1–5]. The exact mechanism by which aspirin exerts its anticancer activity is not clear. However, NSAID drugs are found to block the synthesis of prostaglandins long chain fatty acid compounds that have various functions, including stimulation of cell proliferation and suppression of immune reaction, both of which are linked to tumor progression. Recently, the structural basis of the aspirin activity inferred from the crystal structure of the inactivated prostaglandin H_2 synthetase was reported [6]. If aspirin exhibits anticancer activity, its reaction with cell particles such as nucleic acids, DNA polymerases, and proteins is of great biological importance. In view of the biochemical activities of the aspirin, we were prompted to investigate the aspirin-DNA interaction and to determine drug binding site, sequence specificity, helical stability and DNA secondary structure in aqueous solution. Vibrational spectroscopy (Raman and infrared) is often used to characterize the nature of drug-DNA interaction and to monitor the effects of various drugs on DNA structure [7–9]. Recently, we applied vibrational spectroscopy to analyse the nature of vitamin C complexation with calf-thymus DNA at physiological pH and different drug concentrations [10]. This study allowed us to determine the drug binding sites, the sequence specificity, the DNA secondary structure and the helical stability, in aqueous solution. We believe that vibrational spectroscopy can also be used here, in order to investigate the complex formation between DNA and aspirin and to elucidate the nature of this biologically important drug-DNA interaction.

In this communication, we report the interaction of calf-thymus DNA with aspirin in water/methanol (75 : 25, v/v) solution at pH 7–6 with aspirin/DNA(P) molar ratios r = 1/40 to 2, using FTIR and laser Raman spectroscopic techniques, that have not been previously reported.

2. Materials and methods

Highly polymerized calf-thymus DNA sodium salt (7% Na content) was purchased from Pharmacia and used as supplied. Aspirin was from Aldrich Chemical Co., and used without further purification.

2.1. Preparation of stock solutions

Na-DNA was dissolved to 4% w/w, 0.1 M DNA(phosphate) in 0.1 M NaCl solution at 5°C for 24 h with occasional stirring to ensure the formation of homogeneous solution. Solutions of aspirin 1.2 to 100 mM were also prepared in water/methanol mixture (75:25, v/v) (methanolic solution was used to dissolve aspirin). In the final step, the appropriate amount of drug solution was added dropwise to DNA solution with constant stirring to give the desired drug/DNA molar ratios of 1/40, 1/20, 1/10, 1/4, 1/2, 1 and 2 at a final DNA concentration of 2% w/w or 0.05 M DNA(phosphate). Solution pH was adjusted to 7–6, with 0.1 M NaOH or HCl.

Infrared spectra were recorded on a Bomem DA3-0.02 Fourier Transform infrared instrument, equipped with a nitrogen cooled HgCdTe detector and KBr beam splitter. Solution spectra were taken using AgBr windows with resolution of 2 cm^{-1} and 100 to 500 scans. The H_2O subtraction was carried out as reported [11]. A good subtraction of water was achieved as shown by a flat baseline around 2200 cm^{-1}, where the water combination mode is located. The difference spectra [(DNA solution + aspirin solution) − (DNA solution)] were obtained, using the band at 968 cm^{-1} as internal standard. This band due to the deoxyribose C-C stretching modes, exhibits no spectral changes (intensity or shifting) on drug-DNA interaction and is cancelled, upon spectral subtraction.

Raman spectra were recorded on a DILOR Omars-89 Raman spectrometer, using 514.4 nm line of an argon laser (Spectra-Physics, Model 2020-30). The laser power was 300 mW at the sample. The spectra were typically recorded at 5 cm^{-1} slit width with a 2 s integration time at each 2 cm^{-1} frequency increment. They were routinely background-corrected by subtracting appropriate third-degree polynominal function from original curve. The spectra reproduced here are not smoothed. The Raman difference spectra [(DNA solution +

*Corresponding author.

Abbreviations: asp, aspirin

S0014-5793/96/$12.00 © 1996 Federation of European Biochemical Societies. All rights reserved.
SSDI S0014-5793(96)00093-2

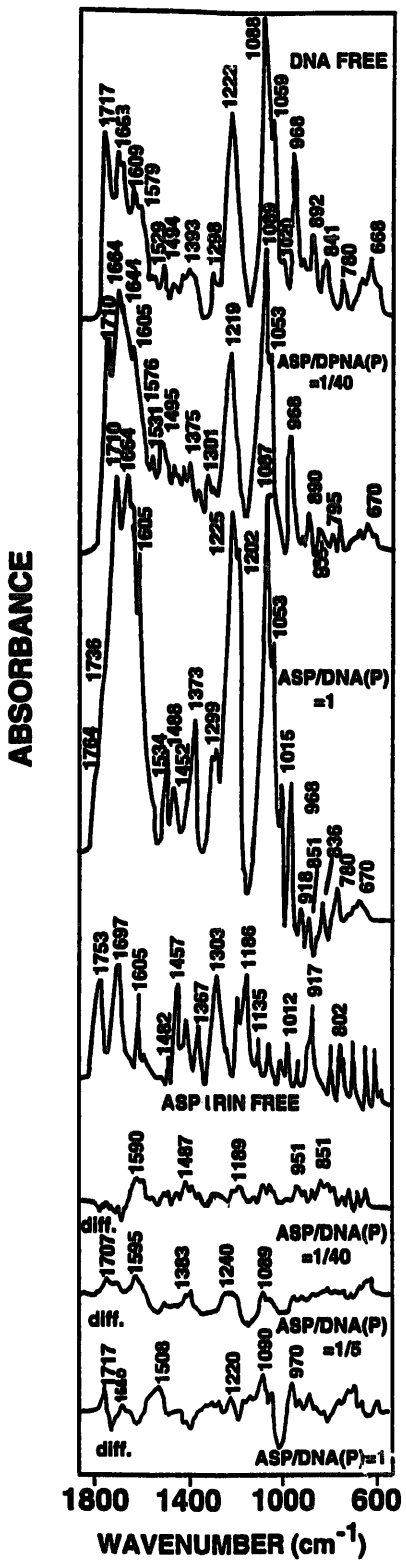


Fig. 1. FTIR spectra (top four curves) and difference spectra (bottom three curves) [(DNA solution + aspirin solution) − (DNA solution)] for the uncomplexed calf-thymus DNA and its aspirin complexes at pH 7–6 with different drug concentrations (drug/DNA(P) molar ratios) in the region of 1800–600 cm^{-1}.

aspirin solution) − (DNA solution)] were produced according to our previous report [12], using the intrense Raman line at 787 cm^{-1} as internal reference. This band due to the coupling of cytosine and phosphodiester modes, exhibits no spectral changes (intensity and shifting) on aspirin-DNA complexation and is cancelled, on spectral subtraction.

The calculated intensity ratios of several prominent DNA in-plane vibrations (normalized against the band at 968 cm^{-1}) were measured as a function of aspirin concentration with error of ± 5%. The results from calculated intensity were used to quantify the amounts of drug-PO_2 interaction versus drug-base binding.

3. Results and discussion

3.1. Aspirin-DNA complexes

At low aspirin concentration (r = 1/40), drug binding is mainly through the backbone PO_2 group and the A-T bases. Evidence for this comes from major intensity increase and shifting of the infrared bands at 1222 cm^{-1} (PO_2 antisymmetric stretch) and at 1663 cm^{-1} (mainly thymine) and 1609 cm^{-1} (mainly adenine) [11–19]. The intensity increase of these vibrations is also associated with the shift of the bands at 1663 to 1664 cm^{-1}, at 1609 to 1605 cm^{-1} and 1222 to 1219 cm^{-1} (Figs. 1 and 2). The calculated intensity ratios of several DNA in-plane vibrations (related to A,T,G,C bases and the backbone PO_2 groups) as a function of aspirin concentration are presented in Fig. 2. The observed spectral changes are due to the drug-DNA interaction via backbone phosphate group and the thymine O-2 and adenine N-7 atoms, that are not normally involved in Watson-Crick hydrogen bonding network. Such interaction does not bring about helix destabilization. It is important to note that, although the band at 1717 cm^{-1} (G,T) shifted towards a lower frequency at 1710 cm^{-1}, no intensity variation is observed for this frequency, upon drug interaction (Figs. 1 and 2, r = 1/40). This is indicative of no major aspirin interaction via G-C base pairs at this stage. The shift of the infrared marker band at 1717 cm^{-1} (G,T) towards a lower frequency is associated with a partial biopolymer conformational conversion, which will be discussed further on.

To support drug binding to the backbone PO_2 and the A-T rich region of the biopolymer, the Raman spectra of the aspirin-DNA complexes are also examined here. The major guanine lines at 1491 cm^{-1} (G) and 1581 cm^{-1} (G), in the Raman spectrum of the uncomplexed DNA [13], exhibited no major spectral changes (shifting or intensity variations) on drug complexation, while the mainly adenine and thymine lines at

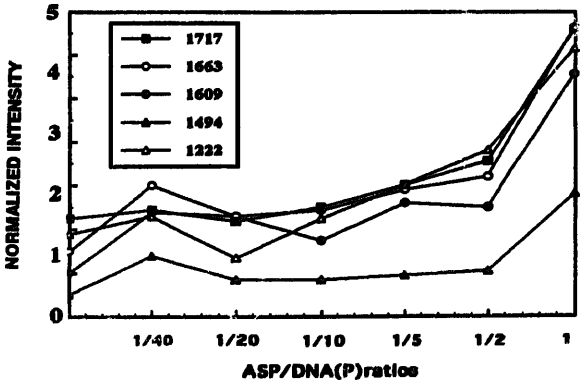


Fig. 2. The calculated intensity ratios of several DNA in-plane vibrations (cm^{-1}) as a function of aspirin concentration (different asp/DNA(P) molar ratios).

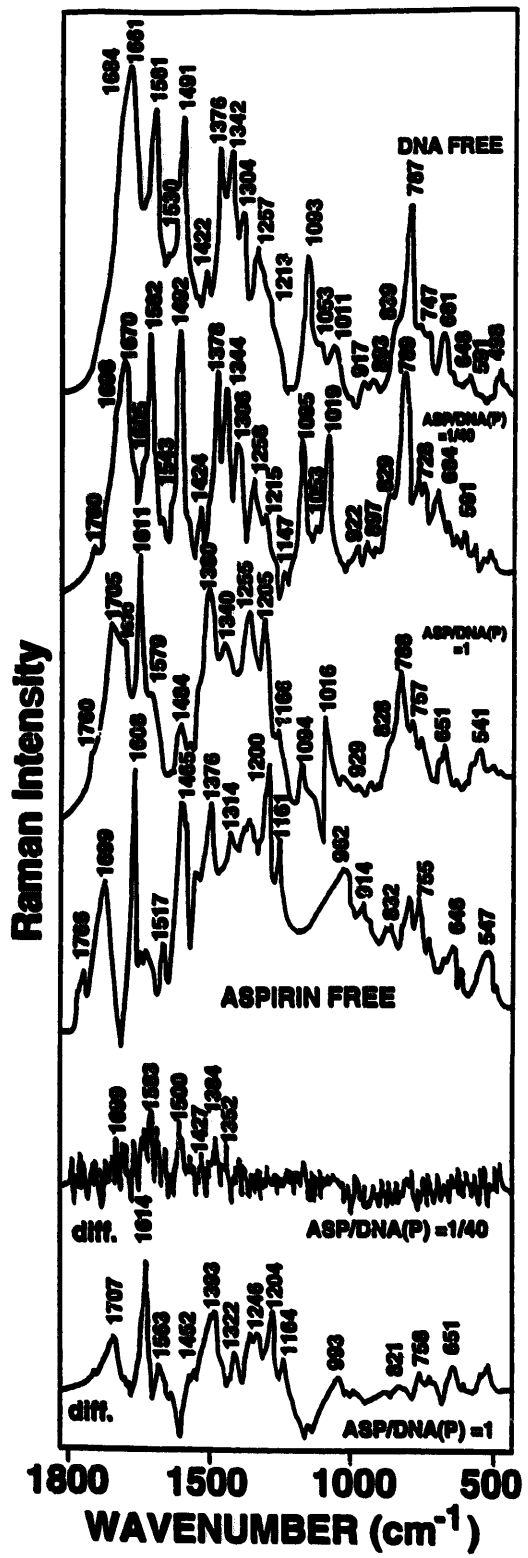


Fig. 3. Laser Raman spectra (top four curves) and difference spectra (bottom two curves) [(DNA solution) + aspirin solution − (DNA solution)] for uncomplexed calf-thymus DNA and its aspirin complexes at pH 7–6 with different drug concentrations (drug/DNA(P) molar ratios) in the region of 1800–500 cm^{-1}.

1342 (A), 1304 (A) 1661 (T) and 1376 cm^{-1} (T,A) shifted towards higher frequencies at 1344, 1306, 1670 and 1376 cm^{-1}, respectively, on drug complexation (Fig. 3, r = 1/40). Similarly, the PO_2 line at 1093 cm^{-1} is observed at 1095 cm^{-1}, in the spectra of the aspirin-DNA complexes (Fig. 3). The observed Raman spectral changes prove the existence of a major drug-DNA interaction through backbone phosphate and the A-T base pairs (H-bonding via H_2O). In the infrared and Raman difference spectra of the drug-DNA complexes with r = 1/40, several weak positive derivative features are observed at 1590 (DNA), 1487 (DNA), 1189 (asp), 951 (asp) and 851 cm^{-1} (asp), that are mainly due to the aspirin anion and DNA vibrational frequencies (Figs. 1 and 3). The calculated intensity ratios of the infrared bands at 1222 cm^{-1} (PO_2 stretch) and at 1663 and 1609 cm^{-1} (T and A vibrations) show almost similar amounts of drug-PO_2 and drug-base bindings (15%) at this stage (Fig. 2).

At r = 1/20, a minor reduction of intensity is observed for DNA in-plane vibrations at 1717, 1663, 1609, 1494 and 1222 cm^{-1} (Fig. 2). The PO_2 band at 1222 cm^{-1} shifted towards a higher frequency at 1225 cm^{-1}, while the IR marker band at 1717 (G,T) shifted to 1710 cm^{-1} (Fig. 1). Similarly, the loss of intensity and shifting of the marker infrared band at 841 cm^{-1} (O-P-O mode) to a lower frequency at 835 cm^{-1} occurred, in the infrared spectra of aspirin-DNA complexes (Fig. 1). The observed spectral changes are attributed to a partial reduction of B-DNA structure towards A-DNA conformation, upon drug complexation [19]. Similarly, the loss of intensity and shifting of the Raman marker line at 839 cm^{-1} (phosphodiester mode) [13] to a lower frequency at 828 cm^{-1} are due to a reduction of the B-DNA structure towards A-DNA formation (Fig. 3). It is important to note, that previous studies have indicated that the oxoanion with strong affinity towards H-bond formation with DNA donor sites or water of hydration, in the grooves of the helix, can stabilize a particular conformation over other structures [16]. Thus, the interaction of the aspirin anion with the water of hydration around DNA bases and the backbone sugar-phosphate moieties alters the activity of water and stabilizes some degree of A-DNA conformation. In a recent study by Loprete and Hartman [20] it is also shown that organic and inorganic anions can alter the secondary structure of the synthetic DNAs in many ways, by altering the water structures around DNA bases and the backbone phosphate groups and stabilizing certain DNA conformation.

At r > 1/20, aspirin interaction with guanine bases occurs. Evidence for this comes from an increase in the intensity of the band at 1717 cm^{-1} which is related mainly to the guanine vibration (Fig. 2). Similar increase in intensity is also observed for the PO_2 band at 1222 cm^{-1} (Fig. 2). However, the mainly cytosine band at 1494 cm^{-1} exhibits no major intensity variations or shifting, upon drug interaction. The observed changes are due to a major drug interaction with the phosphate group and the guanine bases (N-7 site) with no perturbation of the cytosine residues. Evidence for the guanine participation in drug interaction comes also from the shift of the guanine lines at 1491 and 1581 cm^{-1} towards lower frequencies, in the Raman spectra of the aspirin-DNA complexes formed at r > 1/20 (Fig. 3). However, this type of complexation with the guanine bases does not also cause helix destabilization, since no major increase of intensity is observed for the DNA in-plane vibrations [13].

At r > 1/10, drug-DNA interaction continues through ade-

nine, thymine and guanine bases as well as the backbone PO_2 groups with no major participation of the cytosine bases. Evidence for this comes from an intensity increase of the infrared bands at 1717 cm^{-1} (G,T), 1662 cm^{-1} (T,G,A,C), 1609 cm^{-1} (A) and 1222 cm^{-1} (PO_2), while the mainly cytosine bands at 1494 and 1529 cm^{-1} exhibit no intensity variations, upon aspirin interaction (Fig. 2). The positive derivative features observed at 1707, 1660, 1595, 1383, 1240 and 1089 cm^{-1}, in the infrared difference spectra of drug-DNA complexes, are also related to a major intensity increase of the DNA bases and the PO_2 vibrational frequencies, as a result of a strong aspirin-DNA interaction (Fig. 1, r = 1/5). Similarly, the Raman lines at 1661 cm^{-1} (T), 1581 and 1491 cm^{-1} (G), 1376 cm^{-1} (T,A), 1342 and 1304 cm^{-1} (A) and 1093 cm^{-1} (PO_2) exhibit shiftings and intensity changes, while the Raman band at 1257 cm^{-1}, due to cytosine mode [13], shows no alterations on aspirin complex formation (Fig. 3). The calculated intensity ratios of DNA in-plane vibrations, related to the A,T,G and PO_2 modes, show major drug-base binding (30%) with respect to drug-PO_2 interaction (20%) (Fig. 2).

At r = 1/2, drug-DNA binding to cytosine bases also occurs with major alteration of the cytosine vibrational frequencies. Evidence for this comes from a major intensity increase and shifting of the mainly cytosine bands at 1529 to 1534 cm^{-1} and 1494 to 1488 cm^{-1} (Fig. 1,2, r = 1). Similarly, the Raman line at 1257 cm^{-1} (C) gains intensity and shifts towards a lower frequency at 1255 cm^{-1} (Fig. 3, r = 1). The drug-DNA bindings through A-T and G-C base pairs continue up to r=1, where major increase of intensity is observed for the infrared bands at 1717 (G,T), 1663 (T,A,G,C), 1609 (A), 1494 (C) and 1222 cm^{-1} (PO_2) (Fig. 2). The presence of several positive derivative features at 1717, 1660, 1508, 1220 and 1090 cm^{-1}, in the infrared difference spectra of the aspirin-DNA complexes, is also due to a considerable intensity increase of DNA bases and phosphate vibrational frequencies, as a result of drug-DNA interaction (Fig. 1, r = 1). Similarly, a major increase in intensity and shifting of the Raman lines at 1661 cm^{-1} (T) to 1673, 1589 cm^{-1} (G) to 1579, 1491 cm^{-1} (G) to 1484, 1376 cm^{-1} (T,A) to 1380, 1342 cm^{-1} (A) to 1340 and 1257 cm^{-1} (C) to 1255 occurs in the spectra of the aspirin-DNA complexes (Fig. 3, r = 1). These spectral changes are also consistent with the strong positive derivative features observed at 1563, 1452, 1393, 1322, 1246 and 1204 cm^{-1}, in the Raman difference spectra of the drug-DNA complexes (Fig. 3, r = 1). The calculated intensity ratios of the DNA vibrations exhibit an increase in drug-base binding (40%), while the aspirin-PO_2 interaction shows no major alteration (30%), as drug concentration increases (Fig. 2).

At r = 2, the aspirin-DNA interaction causes helix destabilization. Evidence for this comes from major intensity increase of several DNA in-plane vibrations and the shifting of the DNA marker bands at 1717 cm^{-1} (G,T) to 1696, 1663 cm^{-1} (T,G,A,C) to 1656, 1494 cm^{-1} (C,G) to 1484, 1298 cm^{-1} (C,A) to 1306, 1088 cm^{-1} (PO_2) to 1090 (spectra is not shown). Similar partial helix destabilization is also observed for the ascorbate-DNA complexes formed at $r > 1$ [10]. The helix perturbation induced by aspirin is more pronounced than those associated with the ascorbate anion at high drug concentration [10]. However, by comparing the spectral alterations of the calf-thymus DNA in the presence of H^+ and Cu cations (fully melted) and aspirin (partially destabilized), the observed intensity increases of certain DNA in-plane vibrations (marker bands) are larger (fourfold) for proton and copper cations, than those of the aspirin-DNA complexes (twofold) [21,22]. It is worth mentioning, that the reason for the structural dependency of aspirin-DNA complexes on drug concentration is mainly related to different types of drug-DNA complexes, formed (with different DNA binding sites) as aspirin concentration is increased.

The major spectral shiftings observed for the aspirin in-plane vibrations, are also characteristics of drug interaction via anion specific donor sites. The infrared bands at 1760, 1753, 1692, 1561, 1467, 1431, 1345, 1122, 1028 cm^{-1} assigned to the free drug in-plane C=O, O-C-O and C=C, C-O and C-C stretching vibrations [10], exhibited major shiftings, upon DNA complexation (Fig. 1, r = 1). The observed spectral changes are evidence for a major aspirin-DNA interaction through drug C=O, $COOCH_3$ and C-O groups. Similarly, the Raman lines at 1766, 1699, 1608, 1465, 1200 and 962 cm^{-1} related to the aspirin C=O, OCO, C=C, C-O and C-C vibrations exhibited major shiftings in the spectra of drug-DNA complexes (Fig. 3). The observed Raman spectral differences are also characteristics of aspirin-DNA complexation through drug C=O and C-O donor atoms. Similar behaviors were observed in the infrared and Raman spectra of the ascorbate-DNA complexes, where drug binding was through anion C=O, C-O and OH donor atoms [10]. The calculated intensity ratios for DNA bases and the phosphate vibrations show major drug-DNA binding via nucleobases (50%) with respect to the drug-PO_2 interaction (30%) at high aspirin concentration (Fig. 2, r = 1).

In conclusion, the spectroscopic results presented here for the first time, clearly show that at low drug concentration (r = 1/40), aspirin anion binding is mainly through the backbone PO_2 groups and the A-T bases (such as adenine N-7 and thymine O-2 atoms that are not involved in W-C hydrogen bonding network). As drug concentration increases ($r > 1/20$), aspirin binding extends to guanine bases (N-7 site). This type of complexation does not cause helix perturbation. However, at high aspirin content ($r > 1/2$), drug binding to A,T,G,C bases occurs with a partial helix destabilization. Aspirin-DNA complexation causes a partial reduction of the B-DNA structure in favor of A-DNA.

Acknowledgements: This work was supported by a grant from NSERC (Canada).

References

[1] Heath, C.W., Thun, M.J., Greenberg, E.R., Levin, B. and Marnett, J.L. (1994) Cancer 74, 2885–2888 (and references cited therein).
[2] Marnett, L.J. (1992) Cancer Res. 52, 5575–5589.
[3] Paganini-Hill, A.L. (1993) Br. Med. J. 307, 278–279.
[4] Baron, J.A. and Adami, H.O. (1994) Epidemiology 5, 133–134.
[5] Kalgutkar, A.S. and Marnett, L.J. (1994) Biochemistry 33, 8625–8628 (and references cited therein).
[6] Loll, P.J., Picot, D. and Garavito, M. (1995) Nature (structural biology) 2, 637–643.
[7] Morjani, H., Riou, J.F., Riou, J.-F., Nabiev, I., Lavalle, F. and Manfait, M. (1993) Cancer Res. 53, 4784–4790.
[8] Le Gal, J.M., Morjani, H. and Manfait, M. (1993) Cancer Res. 53, 3681–3686.
[9] Sequaris, J.-M., Koglin, E. and Malfoy, B. (1984) FEBS Lett. 173, 95–98.
[10] Neault, J.F., Naoui, M. and Tajmir-Riahi, H.A. (1995) J. Biomol. Struct. Dyn. 13, 387–397.

[11] Alex, S. and Dupuis, P. (1989) Inorg. Chim. Acta 157, 271–281.
[12] Langlais, M., Tajmir-Riahi, H.A. and Savoie, R. (1990) Biopolymers 30, 743–752.
[13] Spiro, T.G. (1987) Biological Applications of Raman Spectroscopy, Wiley, New York.
[14] Stangret, J. and Savoie, R. (1993) J. Mol. Struct. 297, 91–102.
[15] Stangret, J. and Savoie, R. (1992) Can. J. Chem. 70, 2875–2883.
[16] Keller, P.B. and Hartman, K.A. (1986) Spectrochim. Acta 42A, 299–306.
[17] Loprete, D.M. and Hartman, K.A. (1993) Biochemistry 32, 4077–4082.
[18] Starikov, E.B., Semenov, M.A., Maleev, V.Y. and Gasan, A.I. (1991) Biopolymers 31, 255–273.
[19] Brahms, S., Brahms, J. and Pilet, J. (1974) Isr. J. Chem. 12, 153–163.
[20] Loprete, D.M. and Hartman, K.A. (1995) J. Biomol. Struct. Dyn. 13, 57–67.
[21] Tajmir-Riahi, H.A., Ahmad, R., Naoui, M. and Diamantoglou, S. (1995) Biopolymers 35, 493–501.
[22] Tajmir-Riahi, H.A., Neault, J.F. and Naoui, M. (1995) FEBS Lett. 370, 105–108.