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Structural Analysis of DNA Interaction with Spermine - Selenomethionine - Spermine Complex : A Study using Raman and Infrared Spectroscopy

N. Iyandurai and S. Ganesan⁎

Dept. of Physics, Thiruvalluvar Govt. Arts College, Rasipuram, India
⁎Dept. of Physics, Govt. College of Technology, Coimbatore, India
E-mail: ayandurai@yahoo.com, sganesan777@rediffmail.com

Abstract

In the present work, FT Raman and FTIR spectroscopy had been used to extend our knowledge about spermine-DNA, selenomethionine - spermine - DNA and spermine - selenomethionine - spermine - DNA interaction at 1:5 volume ratios. The analysis of FT Raman and FT IR data supported the existence of structural specificities in the interaction. From the observed results, the effect of spermine on DNA is reversed when selenomethionine is added with spermine-DNA complexation. This effect, yet again, appeared to be reversed with the co-administration of spermine with selenomethionine – spermine – DNA complexation. For example, Raman band observed at 1511 cm^{-1} is assigned to an adenine carbon-carbon stretching vibration of pure DNA. This band shifts downward by 3 cm^{-1} in the spectra of spermine - DNA complexation. Similarly, this band shifts upward by 2 cm^{-1} in the spectra of selenomethionine – spermine – DNA complexation and again shifts downward by 3 cm^{-1} in the spectra of spermine - selenomethionine – spermine – DNA complexation.

Keyword: Selenomethionine, spermine, DNA, FT Raman, FTIR.

Introduction

DNA damage is one of major topics of research in cancer biology. In humans, DNA damage has been shown in a variety of genetically inherited disorders, in aging, and in carcinogenesis. Over 74,000 damage incidences occur in DNA per cell per day, mostly by oxidation, hydrolysis, alkylation, radiation, human-made mutagenic chemicals (especially aromatic compounds that act as DNA intercalating agents) or

toxic chemicals that can either directly damage one of the 3 billion bases contained in DNA or create breaks in the phosphodiester backbone that the bases sit on. The result can be mutations in genes, which are, transferred the gene product (protein). If these mutations are in genes that normally control cell proliferation or suppress tumor growth, the cells may start to grow uncontrollably. Cells have therefore developed mechanisms to repair DNA damage but when they stop working efficiently, the number of mutations in our genome increases which can lead to the development of cancer.

Polyamines are small aliphatic polycations that bind with DNA and regulate gene expression and cell proliferation, possibly through regulation of chromation (1-4). The positive charge of polyamines enables them to bind to and modify the negatively charged structures in the cell, such as DNA, proteins, RNA and phospholipids. Historically it was believed that the role of polyamines was as intracellular growth factors, increasing the rate of cell growth and differentiation. But recently, it has been shown that polyamines can also regulate the cell death process known as apoptosis (5). So the polyamines are bivalent regulators of cellular function, promoting cell growth or cell death depending on their environmental signals. Under normal circumstances polyamine concentrations regulate their own biosynthesis and prevent overproduction. Overall, it would appear that imbalances in polyamine metabolism might be an important signal for the cell to undergo apoptosis. Abnormally high polyamine concentrations are well known to be detrimental to cell growth and able to lead to cell death (6). Similarly Polyamine deficiency can under certain circumstances also results in programmed cell death or apoptosis. Although accumulation of polyamine levels appears to be able to trigger apoptosis, decrease in polyamine levels, especially of spermine seems to be a more common feature in apoptosis (7). With high levels of polyamines being so strongly associated with rapid proliferation and growth of tumors, it is understandable that great effort has been placed on designing inhibitors of polyamine synthesis and polyamine analogs and testing their ability to restrict growth of tumor and cancer cells. Similarly Polyamine deficiency may be achieved by treating cells with specific inhibitors of the polyamine biosynthetic enzymes.

Selenium is an essential trace mineral for the human body; primarily it acts as an effective antioxidant preventing harmful cell damage. In nature, however, selenium is found bound to amino acids, for example methionine. The selenium amino acid complex is easily assimilated and superior to other types of selenium supplementation such as sodium selenite and artificially selenized yeast. The most efficacious and safest way of supplementing our diet with selenium is not the inorganic salt form, but the organic forms, like selenium yeast and l-selenomethionine. Too high selenium is toxic. Prospective studies and recent intervention trials suggest that the risk of some cancers, including respiratory tract cancers, may be inversely related to selenium intake, and this is supported by strong experimental evidence with chemical-induced animal cancer models (8). In general, there is a correlation between the effectiveness of selenium compounds (selenomethionine) as chemopreventive agents *in vivo* and their ability to inhibit cell growth and induce apoptosis *in vitro*. Selenium metabolism and polyamine biosynthesis are linked in that they both require S-adenosylmethionine

as a cofactor (9).

Claire Redman, et al. 1997 (9) found that selenomethionine inhibited tumor growth (both in A549 lung and HT29 colon cancer cells) in a dose dependent manner. Simultaneously the polyamine content of A549 and HT29 cancer cell lines was degreased at doses that inhibited 50% of normal growth. These polyamine-depleted cells exhibit a high degree of chromosomal aberrations (10) and eventually stop dividing (11). Selenomethionine treatment induced apoptosis in both cancer cell lines. Exogenous spermine administration, which replenishes intracellular polyamine levels, prevented selenomethionine-induced apoptosis. Selenomethionine administration to the cancer cell lines increased the number of cells in the metaphase. This cell cycle effect appeared to be reversed with the co-administration of selenomethionine and spermine.

Spermine depletion and other polyamine decreases were observed in selenomethionine-treated A549 and HT29 cells by Microscopy and Flow cytometric analysis of cell cycle methods. They have provided information on the overall structure of the cell. However, the determination of specific sites of interaction through such methods is very indirect.

In view of this, the objective of the present work is to characterize the structural changes that DNA undergoes in the presence of spermine, selenomethionine – spermine complex and spermine - selenomethionine – spermine complex**.** One of the methods of promise to provide information on binding sites, stacking interactions and conformation, is vibrational spectroscopy, particularly FT Raman and FTIR spectroscopy. Here the FT Raman and FTIR spectroscopy have been used to analyze the effect of spermine on DNA, selenomethionine on spermine - DNA complexation and spermine on selenomethionine - spermine - DNA complexation.

Materials and Methods

Materials

Highly polymerized calf-thymus DNA sodium salt (6.2% sodium content, 13% H2O content), spermine and selenomethionine were purchased from Sigma Chemical Co. DNA was deproteinated by the addition of $CHCl_3$ and isoamyl alcohal in NaCl solution.

Sodium-DNA was dissolved in 50mM NaCl (pH 7.20) at 5°C for 24 h with occasional stirring to ensure the formation of a homogeneous solution.

The appropriate amount of spermine (50mM) was prepared in distilled water and added drop wise to the DNA solution to attain the desired spermine - DNA (1:5), volume ratios. Similarly the appropriate amount of selenomethionine (50mM) was prepared in distilled water and added drop wise to the spermine - DNA (1:5) to attain the desired selenomethionine - spermine - DNA (1:1:5), volume ratios. Finally, spermine was added drop wise to the selenomethionine - spermine - DNA (1:1:5) to attain the desired spermine - selenomethionine - spermine - DNA (1:1:1:5) volume ratios. The pH of all the solutions was adjusted at 7.

Methods

FT Raman measurements

FT Raman spectra were recorded in a Bruker Equimox 55 FT spectrometer supplied with a Raman module. Spectra are applied at the spectral resolution of $2cm^{-1}$, using excitation radiation wave number at 1064 nm from Nd-YAG laser working at 500 mW. We used a standard quartz cell for liquids (1-cm section) where approximately 1 ml of the solutions was placed. Backscattering collection of the Raman radiation was performed using a mirror behind the cell and minimum of 2000 scans were accumulated in all case to enhance the signal-to-noise ratios. Raman spectra were recorded between 600 and 1800 cm^{-1}. The Raman spectra of spermine- DNA, selenomethionine -spermine - DNA and spermine – selenomethionine – spermine – DNA complexes at higher volume ratios (1:5, 1:1:5, 1:1:1:5) are recorded.

FTIR measurements

Infrared spectra were recorded with a FTIR spectrometer (Impact 420 model) equipped with deuterated triglycine sulfate detector and KBr beam splitter, using AgBr windows. Interferograms were accumulated over the spectral range 600-1800 cm^{-1} with a nominal resolution of $2cm^{-1}$ and a minimum of 100 scans. The water subtraction was carried out using 0.1 M NaCl solution at pH 7.0 ± 0.2 as a reference. The infrared spectra spermine- DNA, selenomethionine -spermine - DNA and spermine – selenomethionine – spermine – DNA complexes at higher volume ratios (1:5, 1:1:5, 1:1:1:5) are recorded.

Results and Discussion

Analysis of FT Raman spectroscopic data

The FT Raman spectra of calf-thymus DNA, spermine, selenomethionine, spermine-DNA complex (1:5 volume ratio), selenomethionine – spermine – DNA complex (1:1:5 volume ratio) and spermine - selenomethionine – spermine – DNA complex (1:1:1:5 volume ratio) are displayed in figure I and Table I shows the relevant wave numbers with intensity (given in brackets) for the complete range of concentrations studied. Generally, polycations induce condensation and precipitation of the negatively charged DNA strands and this effect is further enhanced by further addition of positive charge of the counterion (12). In our experiment, we are using ornithine-derived polyamine i.e., spermine as a polycation at physiological pH. However DNA precipitation with tetravalent spermine occurs at a low concentration than for trivalent spermidine. When we increase the spermine concentration, which is greater than 10 mM, DNA gets precipitated in our experimental condition. Spermine solutions ranged between 1-7.5 mM are physiological concentrations. At the same time DNA strands of calf-thymus containing small amount of polyamines in adenine, guanine and cytosine moiety.

The FT Raman spectra of the spermine-DNA, selenomethionine-spermine-DNA and spermine - selenomethionine-spermine-DNA complex show wave number shifts, indicating that interactions are present without condensation.

Analysis of FTIR spectroscopic data

The FTIR spectra of calf-thymus DNA, spermine, selenomethionine, spermine-DNA complex (1:5 volume ratio), selenomethionine – spermine – DNA complex (1:1:5 volume ratio) and spermine - selenomethionine – spermine – DNA complex (1:1:1:5 volume ratio) are displayed in figure II and Table II shows the relevant wave numbers with intensity (given in brackets) for the complete range of concentrations studied. The FTIR spectra of the spermine-DNA, selenomethionine-spermine-DNA and spermine - selenomethionine-spermine-DNA complex show wave number shifts, indicating that interactions are present without condensation.

Interpretation of FT Raman spectra of solutions in water

FT Raman spectra of DNA have been comprehensively studied in recent years, although not all the bands have been indisputably assigned up to now. In Raman spectra, bands mainly arises from both base and phosphate vibrations, together with some deoxyribose contributions.

The region between 1700 and 1200 cm^{-1} in the Raman spectrum is clearly dominated by base vibrations, which involves the stretching modes of aromatic rings, the carbon -oxygen double bond stretching and some methyl bending vibrations, and the Raman band observed around 1400-1450 cm^{-1} is assigned to methylene scissoring modes of the deoxyribose units (13).

The Raman band observed at 1666 cm^{-1} corresponds to C=O stretching mode and it has been largely assigned to the C2=O2 bond of thymine residues (14). Thymine O2 atoms are a reputed site of interaction of DNA at the minor groove because they are not involved in Watson-Crick hydrogen bonds. This band does not show any shift in spermine – DNA, selenomethionine - spermine - DNA and spermine - selenomethionine - spermine - DNA complexation. Contradictory, opposite behavior is observed at the Raman band at 1487 cm^{-1} of DNA, which has been assigned to guanine-N7 reactive site (15). It shifts downward by 5 cm^{-1} in the Raman spectra of spermine - DNA complex, shifts upward by 4cm^{-1} in the Raman spectra of selenomethionine - spermine-DNA complex and again shifts downward by 4cm^{-1} in the Raman spectra of spermine - selenomethionine - spermine-DNA complex.

Raman bands of spermine at 1452 and 1475 cm^{-1} have been assigned to an interaction site with guanine - N7 reactive site (16). Raman band at 1511 cm^{-1} is assigned to an adenine carbon-carbon stretching vibration (16). This band shifts downward by 3 cm^{-1} in the spectra of spermine-DNA complex, shifts upward by 2 cm^{-1} in the spectra of selenomethionine - spermine – DNA complex and again shifts downward by 3 cm^{-1} in the Raman spectra of spermine - selenomethionine - spermine-DNA complex.

Raman band at 1575 cm^{-1} has been assigned to a purine vibration (17), its behaviour discriminates selenomethionine – spermine and spermine – selenomethionine - spermine, for which this band does not show major frequency shifts. The Raman band measured at 1463 cm^{-1} in the Raman spectrum of DNA has been assigned to the deoxyribose moieties (18). They correspond to methylene bending modes, although they should also have some contributions from adenine vibrations. Interpretation of their wave number shifts is, nevertheless, troublesome for

solutions at the highest spermine concentrations, because they also contain methylene groups. Similarly no major shift is observed in selenomethionine - spermine – DNA and spermine - selenomethionine - spermine - DNA complexes.

Additional evidence of spermine – DNA, selenomethionine – spermine – DNA and spermine - selenomethionine – spermine – DNA interactions by the bases can be obtained from the DNA bands at 1341 cm^{-1} (adenine and guanine), 1301 cm^{-1} (adenine), 1259 cm^{-1} (adenine and cytosine) and 1176cm^{-1} (thymine and cytosine) (19). All of them shift up to several cm^{-1} in the spectra of spermine-DNA complex, shifts down to several cm^{-1} in the spectra of selenomethionine -spermine - DNA complex and again shift up to several cm^{-1} in the spectra of selenomethionine – spermine – DNA complex.

Raman spectrum of DNA between 1100 and 800 cm^{-1} is dominated by two intense bands, namely, at 1088 & 797 cm^{-1}, which have been assigned to the symmetrical stretching vibrations of the phospodioxy (PO_2^-) & phospodiester (P-O-P) moieties respectively (20). In addition, a second phospodiester (P-O-P) peak is measured at 840 cm^{-1}, which is considered as one of the DNA conformational marker bands (20).

The O-P-O marker band at 840 cm^{-1} desires to be discussed independently. This band is very perceptive to both conformation and base sequence (20). As a result, for native DNA, it usually appears as a broad band that cannot be split by deconvolution procedures. For the spermine – DNA, selenomethionine – spermine – DNA and spermine - selenomethionine – spermine – DNA Raman spectra, some deviations are observed with respect to the wave number measured for calf-thymus DNA. However, they do not let to conformational transitions A↔B↔Z in any case. In our opinion, the observed shifts are due to small conformational distortions originated by the molecular interaction.

Raman band at 896cm^{-1} have been assigned to stretching vibrations of the deoxyribose rings for DNA (21). Their shifts upon spermine complexation, selenomethionine –spermine and spermine - selenomethionine – spermine complexations indicate contribution of the sugar moieties in the interaction. Bands between 650 and 800 cm^{-1} in the Raman spectra of the DNA have been assigned to the vibrational bending modes of the nucleic bases. Their shifts upon spermine complexation, preferential link to guanine residues can be inferred for spermine, in agreement with the standard discussion for the stretching modes of the bases, between 1700 abd 1200 cm^{-1}.

The observation of the NH_3^+ stretching and bending vibrations for any solute of a water solution is not an easy task because they lie close to the related O-H vibrations of the solvent molecules at 3000 and 1700cm^{-1} respectively. At higher spermine concentrations, it goes to lower wave numbers as a probe of the active participation of these groups in the interactions. However, the NH_3^+ groups have a third type of vibrational mode, generally called the rocking mode (22), which gives rise to one or two Raman bands around 1100 cm^{-1}. It has been observed at 1065 cm^{-1} for spermine. At higher spermine concentrations it goes to a lower wave numbers as a probe of the active participation of these groups in the interactions.

The Raman band at 1481cm^{-1} of selenomethionine is assigned to CO_2^- symmetric stretching mode vibration and at 2565cm^{-1} of selenomethionine is assigned to

asymmetric mode vibration (which is not shown in the spectra). Similarly the band at 1076cm^{-1} is assigned to NH_3^+ group of selenomethionie. These results show that selenomethionine is present in the form of zwitterions similar to amino acids (23). These frequencies go to the lower wavenumber shift at higher selenomethionine concentrations.

Interpretation of FTIR spectra of solutions in water

At all the complexes studied, the interaction of DNA is mainly due to bases. Evidence for this comes from the changes in the intensity and shifting of the bands in the region 1550 - 1800 cm^{-1}, which is assigned to in-plane DNA vibrational frequencies (24). FTIR band at 1718 cm^{-1} is assigned to in-plane stretching vibrations of double bond of guanine (C7=N) located at the major groove (24). This band is shifted downward by 8cm^{-1} in the spectra of spermine - DNA complexes, shifted upward by 6 cm^{-1} in the spectra of selenomethionine – spermine – DNA complexes and again shifted downward by 6 cm^{-1} in the spectra of spermine - selenomethionine – spermine – DNA complexes.

IR band at 1608 cm^{-1} is assigned to in-plane stretching vibrations of double bond of adenine (C7=N) (24). This band shifts downward by 4cm^{-1} in the spectra of spermine - DNA complexes, shifts upward by 3 cm^{-1} in the spectra of selenomethionine – spermine - DNA complexes and again shifted downward by 4 cm^{-1} in the spectra of spermine - selenomethionine – spermine – DNA complexes.

IR band at 1664 cm^{-1} is assigned to in-plane stretching vibrations of double bond of thymine (C2=O) (25). This band shifts downward by 2cm^{-1} in the spectra of spermine - DNA complexes, shifts upward by 1 cm^{-1} in the spectra of selenomethionine – spermine - DNA complexes and again shifts downward by 2 cm^{-1} in the spectra of spermine - selenomethionine – spermine – DNA complexes.

A major increase in the intensity was mainly observed for a guanine band at 1718 cm^{-1}, thymine band at 1664 cm^{-1}, and adenine band at 1608 cm^{-1}. These intensity changes also with band shift are due to the interaction of spermine – DNA, selenomethionine – spermine - DNA and spermine – selenomethionine – spermine – DNA complexes.

FTIR band at 1578 cm^{-1} is assigned to in-plane C8=N7 stretching vibrations of purine ring (mainly guanine residue) (25). This band is shifted downward by 2cm^{-1} in the spectra of spermine-DNA complexes, shifted upward by 1 cm^{-1} in the spectra of selenomethionine – spermine - DNA complexes and again shifted downward by 1 cm^{-1} in the spectra of spermine - selenomethionine – spermine – DNA complexes. This band is evidence and pinpointing of a major spermine, selenomethionine - spermine and spermine – selenomethionine – spermine complexation with the guanine N7 atom.

Other DNA vibrational frequencies in the region 1550-1250 cm^{-1} showed minor spectral changes upon spermine, selenomethionine-spermine and spermine – selenomethionine – spermine complexation. The band at 1492cm^{-1} is assigned largely to the cytosine residue (25,26), which shows no major shifting and its intensity did not change significantly at any spermine, selenomethionine-spermine and spermine – selenomethionine – spermine complexes. Thus the possibility of an interaction

between spermine and cytosine, selenomethionine-spermine and cytosine, and, spermine – selenomethionine – spermine and cytosine is very less.

The FTIR spectra of DNA in the region1250-1000 cm^{-1} are assigned to backbone phosphate group vibrations. Mainly the band at 1224 cm^{-1} and 1088 cm^{-1} are assigned to the asymmetric and symmetric stretching vibrations of the PO_2^- groups (26). The decrease in the intensity and the band shifts upward to 2 cm^{-1}, the increase in the intensity and the band shifts downward to 2 cm^{-1} and again the decrease in the intensity and the band shifts upward to 3 cm^{-1} of the asymmetric PO_2^- at 1224 cm^{-1} reflects the interaction of spermine, selenomethionine – spermine and spermine – selenomethionine – spermine complexes with the oxygen atoms of the backbone phosphate groups. Spermine strongly interacts with the guanine and adenine - N7 sites as supported by the shifts of the bands at 1717 and 1609 cm^{-1}. The similar effect is observed for the selenomethionine – spermine and spermine - selenomethionine – spermine complexes.

FTIR band at 836cm^{-1} is assigned for the sugar – phosphodiester mode, which is considered as a major marker band for the DNA conformation (26). Minor spectral changes were observed for the marker bands in the spectra of spermine – DNA, selenomethionine - spermine-DNA and spermine - selenomethionine – spermine – DNA complexes. However, these minor changes do not lead to conformational transitions such as B↔A↔Z.

The FTIR spectrum of selenomethionine shows doublet at 1587 (CO_2^- asym.) and 1404 cm^{-1} (CO_2^- sym.) in contrast to single band nearly at 1710 cm^{-1}, which is assigned for carboxylic acid groups. These results show that selenomethionine is present in the form of zwitterions similar to amino acids (23). These frequencies go to the lower wavenumber shift at high selenomethionine concentrations.

Discussion

The results obtained from Raman and FTIR spectroscopic study can be discussed in terms of preferential sites of binding between spermine – DNA, selenomethionine – spermine - DNA and spermine - selenomethionine – spermine - DNA complexes, which can be compared with interaction models proposed on the basis of different biochemical, physical and chemical techniques (27). The interaction between spermine and DNA has been widely studied in the past by using different experimental and theoretical techniques and several models have been discussed (28,29). These models however are often contradictory, and the question concerning the spermine - DNA remains troublesome. The observed shifts for DNA guanine-N7 Raman band at 1487 cm^{-1} and FTIR band at 1718cm^{-1} upon spermine addition, selenomethionine-spermine and spermine – selenomethionine – spermne complexes indicate interaction by the major groove, whereas the thymine-O_2 Raman band at 1666cm^{-1} and FTIR band at 1664cm^{-1} does not appreciably shift.

The polyamine molecule is placed across the major groove with three non-sequence-dependent attachment possibilities: the phosphate moieties for the two outer spermine NH_3^+ groups and a purine - N7 atom for an inner NH_2^+ group. The second NH_2^+ group can interact either with thymine O4 or with other purine N7 atoms,

depending on the base sequence; interaction with cytosine residues are, in our opinion, less credible. This conclusion agrees with reported studies about sequence dependence on the spermine DNA interaction, in which significant differences were proposed between A-T and G-C pairs. Similarly, in our opinion; the selenomethionine molecule attracts the spermine molecules otherwise placed across the major groove of the DNA, so that the effect of spermine on DNA is abridged. Hence the wavenumber of DNA go to the higher shift when selenomethionine is further added with spermine - DNA complexes. The CO_2^- of selenomethionine attracts the NH_3^+ groups of spermine otherwise phosphate groups of DNA. N-H……N interaction between selenomethionine and spermine or selenomethionine with bases of DNA is possible in our case. From the experimental findings, it is clear that spermine-DNA interaction is appeared to be reversed when selenomethionine is added with spermine-DNA complexes. On the other hand, when spermine is further added with selenomethionne – spermine – DNA complexes, the effect of selenomethionine – spermine complex on DNA is upturned.

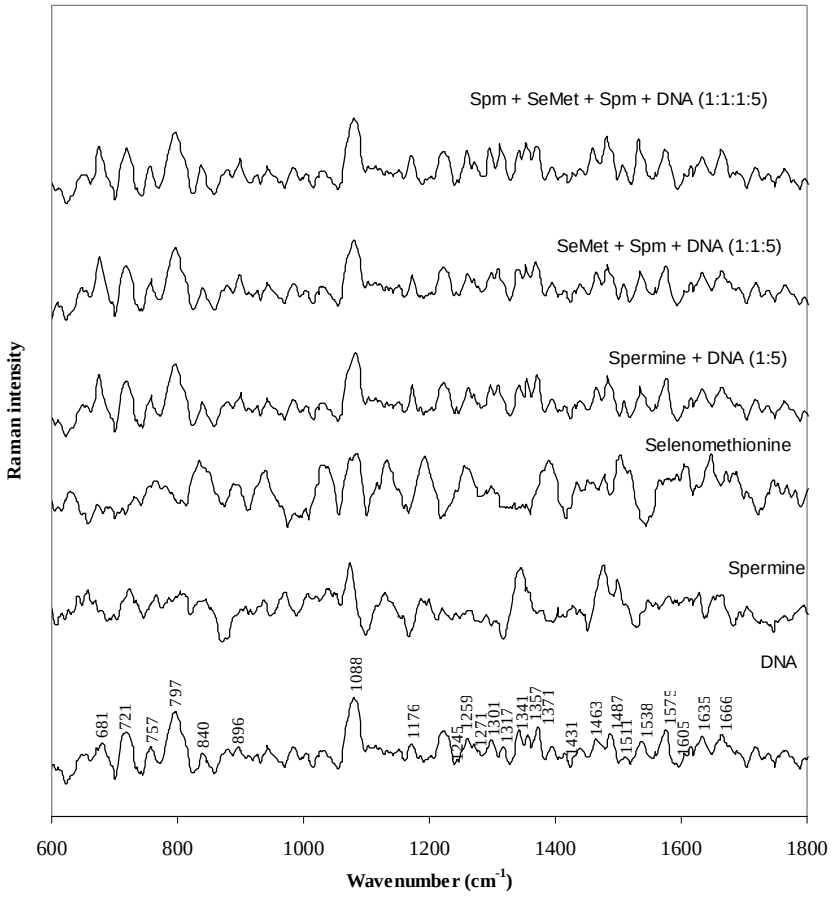

Figure I. FT Raman spectrum of DNA, spermine, selenomethionine, spermine with DNA (1:5 ratio), selenomethionine – spermine complex with DNA (1:1:5 ratio) and spermine – selenomethionine – spermine complex with DNA (1:1:1:5 ratio).

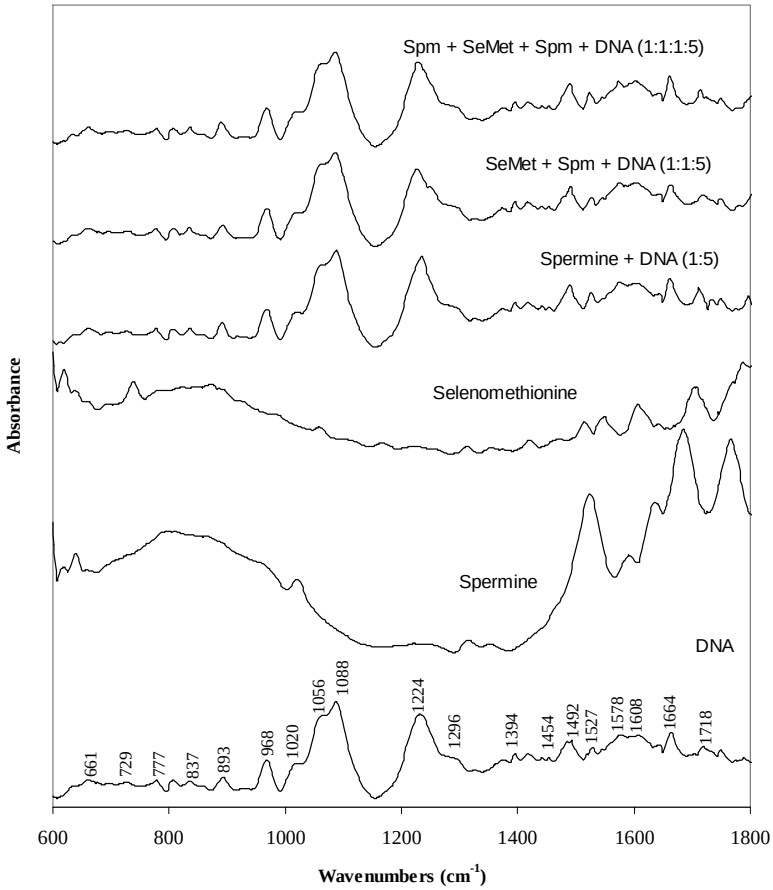

Figure II. FTIR spectrum of DNA, spermine, selenomethionine, spermine with DNA (1:5 ratio), selenomethionine – spermine complex with DNA (1:1:5 ratio) and spermine – selenomethionine – spermine complex with DNA (1:1:1:5 ratio).

Table I. Wave numbers (intensity is given in bracket) for the DNA, spermine – DNA (1:5 ratio), selenomethionine – spermine – DNA (1:1:5) and spermine - selenomethionine – spermine – DNA (1:1:1:5) complexes studied using FT Raman spectroscopy.

DNA	Spermine + DNA (1:5)	SeMet + Spermine + DNA (1:1:5)	Spermine + SeMet + spermine + DNA (1:1:1:5)	Assignments
681(6.2)	676(8)	680(8)	677(7.6)	Guanine
721(7)	721(7.1)	721(7.4)	720(7.5)	dA (C1-N9 str.)
757(5.8)	757(5.9)	757(6.2)	756(6)	Thymine
797(8.8)	797(8.9)	797(8.8)	797(8.8)	OPO sym. str. mode
840(5.4)	837(5.6)	839(5.5)	838(6)	OPO asym. str. mode
896(5.9)	899(6.4)	897(6.4)	899(6.6)	Deoxyribose

1088(10)	1088(9.8)	1088(9.6)	1088(10)	PO^-_2 str.
1176(5.9)	1173(7.1)	1175(6.4)	1173(6.9)	Thymine, Cytosine
1245(4.8)	1244(5.2)	1245(5.2)	1245(4.8)	dC (in-plane ring str.)
1259(6.6)	1262(6.9)	1260(6.6)	1261(7.2)	Adenine, Cytosine
1271(6.1)	1270(6.2)	1271(6.1)	1270(6.3)	dC (in-plane ring str.)
1301(6.3)	1296(7.1)	1300(6.7)	1297(7.5)	Adenine
1317(5.9)	1310(7.1)	1315(7)	1313(7.9)	dG (imidazole ring
1341(7.3)	1340(7)	1341(7.1)	1340(7.3)	coupled with C8-N9
1357(6.9)	1354(7.2)	1356(7.3)	1353(8)	str.)
1371(7.6)	1369(7.9)	1370(7.6)	1369(7.5)	Purine str.
1431(5.1)	1430(5.4)	1431(5.3)	1430(5.4)	dG (C2=N3-C4=C5-
1463(6.4)	1462(6.5)	1462(6.8)	1460(7.5)	N7=C8 triene moiety)
1487(7)	1482(7.9)	1486(7.3)	1482(8.5)	Thymine (CH3),
1511(5)	1508(5.8)	1510(5.8)	1507(6.1)	Purine
1538(6.3)	1535(6.6)	1537(6.8)	1534(8.2)	dT (C5-CH3
1575(7)	1574(7.1)	1575(7.4)	1574(7.4)	deformation)
1605(5.3)	1605(5.3)	1605(5.3)	1605(5.3)	Deoxyribose
1635(6.7)	1634(6.7)	1635(6.7)	1634(6.7)	Guanine (N7)
1666(6.9)	1665(7)	1666(6.8)	1664(7.4)	Adenine
				dG (imidazole ring
				and C6 moiety)
				Purine str.
				dC (ring str.)
				dC (carbonyl str.
				mode)
				Thymine (O2)

Table II. Wave numbers (intensity is given in bracket) for the DNA, spermine – DNA (1:5 ratio), selenomethionine – spermine – DNA (1:1:5) and spermine - selenomethionine – spermine – DNA (1:1:1:5) complexes studied using FTIR spectroscopy.

DNA	Spermine + DNA (1:5)	SeMet + Spermine + DNA (1:1:5)	Spermine + SeMet + spermine + DNA (1:1:1:5)	Assignments
837(2.5)	836(2.6)	837(2.6)	836(2.7)	Deoxyribose, B-
893(2.8)	891(3.2)	892(2.9)	890(3.2)	marker
968(4.4)	968(4.4)	968(4.4)	968(4.4)	Deoxyribose, B-
1056(8.4)	1055(8.3)	1056(8.4)	1055(8.1)	marker
	1087(9.7)	1088(9.7)	1088(9.7)	C-C deoxyribose
1088(10)	1226(8.6)	1224(8.1)	1227(8.8)	stretching
1224(8.4)	1490(6.8)	1491(6.7)	1490(6.8)	C-O deoxyribose
1492(6.3)	1525(6)	1527(5.5)	1525(6)	stretching

1527(5.6) 1578(6.9) 1608(6.8) 1664(7) 1718(5.7)	1576(7) 1604(7.1) 1662(7.3) 1710(6.5)	1577(6.9) 1607(6.9) 1663(6.7) 1716(5.7)	1576(7) 1603(7.2) 1661(7.5) 1710(6.2)	PO_2^- symmetric stretching PO_2^- asymmetric stretching In-plane vibration of cytosine In-plane vibration of cytosine and guanine Purine stretching (N7) Adenine (C7=N stretching) Tymine (C2=O stretching) Guanine (C7=N stretching)

Abbreviation

Spm – spermine, SeMet – selenomethionine, dG – guanine, dA – adenine, dC – cytosine, dT – thymine.

References

[1] C W Tabor, and H Tabor, 1984, Polyamines, Annual Review of Biochemistry, doi 10.1146/annurev.bi.53.070184.003533, v53, pp 749-790.

[2] Anthony E. Pegg., 1988, Polyamine Metabolism and Its Importance in Neoplastic Growth and as a Target for Chemotherapy1, Cancer Research, v48, pp 759-774.

[3] Olle Heby, 1981, Role of Polyamines in the Control of Cell Proliferation and Differentiation, Differentiation, doi 10.1111/j.1432-0436.1981.tb01123.x, v19, pp1-20.

[4] Alexander Tkachenko, Larisa Nesterova, Michael Pshenichnov, 2004, The role of the natural polyamine putrescine in defense against oxidative stress in Escherichia coli, Archives of Microbiology, doi 10.1007/s002030100301, v176, pp155-157.

[5] Wayne E. Criss. 2003, A Review of Polyamines and Cancer, Turkish Journal of Medical Sciences, v33, pp195-205.

[6] He Y., Suzuki T., Kashiwagi K. and Igarashi K, 1994, Antizyme Delays the Restoration by Spermine of Growth of Polyamine-Deficient Cells through Its Negative Regulation of Polyamine Transport, Biochemical and Biophysical Research Communications, ISSN 0006-291X, v203, pp608-614.

[7] Takeshi Nitta, Kazuei Igarashi, Atsuya Yamashita, Mikio Yamamoto and Naoki Yamamoto, 2001, Involvement of Polyamines in B Cell Receptor–Mediated Apoptosis: Spermine Functions as a Negative Modulator, Experimental cell Res., doi 10.1006/excr.2001.5177, v256, pp174-183.

[8] Aurnab Ghose, Janis Fleming, Karam El-Bayoumy and Paul R. Harrison, 2001, Enhanced Sensitivity of Human Oral Carcinomas to Induction of Apoptosis by Selenium Compounds, Cancer Research, v61, pp7479-7487.

[9] C Redman, MJ Xu, YM Peng, JA Scott, C Payne, LC Clark and MA Nelson, 1997, Involvement of polyamines in selenomethionine induced apoptosis and mitotic alterations in human tumor cells, Carcinogenesis, v18, pp1195-1202.

[10] Pohjanpelto P, Holtta E, Janne OA, Knuutila S, Alitalo K., 1985, Amplification of ornithine decarboxylase gene in response to polyamine deprivation in Chinese hamster ovary cells, J. Biol. Chemistry, v260, pp 8532-8537.

[11] Moment PS, Bohlen P, McCann PP, Bey P, Schuber F, Tardif C., 1976, Alpha-methyl ornithine, a potent competitive inhibitor of ornithine decarboxylase, blocks proliferation of rat hepatoma cells in culture, Proc. Natl Acad. Sci. USA., v73, pp1626-1630.

[12] Leonard C. Gosule and John A. Schellman, 1976, Compact form of DNA induced by spermidine, Letters to Nature, doi 10.1038/259333a0, v259, pp 333-335.

[13] Wei Xie, Yong Ye, Aiguo Shen, Li Zhou, Zhaowen Lou, Xiaohua Wang and Jiming Hu., 2008. Evaluation of DNA-targeted anti-cancer drugs by Raman spectroscopy, Vibrational Spectroscopy, doi 10.1016/j.vibspec.2008.03.003, v47, pp119-123.

[14] Ramazan Kizil, Joseph Irudayaraj, 2007. Rapid evaluation and discrimination of γ-irradiated carbohydrates using FT-Raman spectroscopy and canonical discriminant analysis, Journal of the Science of Food and Agriculture, doi 10.1002/jsfa.2830, v87, pp1244-1251.

[15] Denson, Stephen C., Pommier, Carolyn J. S., Denton, M. Bonner., 2007, The Impact of Array Detectors on Raman Spectroscopy, Journal of Chemical Education., ISSN-0021-9584, v84, pp 67-74.

[16] Grace Ferreira Ghesti, Julio Lemos de Macedo, Ines Sabioni Resck, Jose Alves Dias, and Silvia Claudia Loureiro Dias., 2007, FT-Raman Spectroscopy Quantification of Biodiesel in a Progressive Soybean Oil Transesterification Reaction and Its Correlation with ^{1}H NMR Spectroscopy Methods, Energy Fuels., doi 10.1021/ef060657r S0887-0624(06)00657-8 , v21 (5), pp2475–2480.

[17] Leonardo M. Moreira, Landulfo Silveira Jr., Fabio V. Santos, Juliana P. Lyon, Rick Rocha, Renato A. Zangaro, Antonio Balbin Villaverde, Marcos T.T. Pacheco, 2008, Raman spectroscopy: A powerful technique for biochemical analysis and diagnosis, Spectroscopy, doi 10.3233/SPE-2008-0326, v 22, pp1-19.

[18] W. Kiefer, 2007. Recent Advances in linear and nonlinear Raman spectroscopy I, Journal of Raman Spectroscopy, doi 10.1002/jrs.1902, v38, pp1538-1553.

[19] Bruce Chase, 2007. FT-Raman Spectroscopy: A Catalyst for the Raman Explosion? Journal of Chemical Education, v84, pp75-80.

[20] S. Sanchez-Carrasco, J.G. Delcros, A.A. Moya-Garcia, F. Sanchez-Jimenez and F.J. Ramirez, 2008, Study by optical spectroscopy and molecular dynamics of the interaction of acridine – spermine conjugate with DNA, Biophysical Chemistry, doi:10.1016/j.bpc.2007.12.003 , v133, pp54-65.

[21] P. O. Andrade, R. A. Bitar, K. Yassoyama, H. Martinho, A. M. E. Santo, P. M. Bruno and A. A. Martin., 2007. Study of normal colorectal tissue by FT-Raman spectroscopy, Analytical and Bioanalytical Chemistry, doi 10.1007/s00216-006-0819-1, v387, pp1643-1648.

[22] Ruiz – Chica A. J. Medina M. A. Sanchez – Jimenez F. Ramirrez F. J., 2004, On the interpretation of Raman spectra of 1-aminooxy-spermine/DNA complexes, Nucleic acids research, v32, pp579-589.

[23] Kalsi, P.S., 2004, Spectroscopy of organic compounds, New Age International Limited Publishers, ISBN 81-224-1543-1, pp180-181.

[24] Shohreh Nafisi, Mehrdad Hashemi, Mehdi Rajabi, and Heidar Ali Tajmir-Riahi, 2008, DNA Adducts with Antioxidant Flavonoids: Morin, Apigenin, and Naringin, DNA and Cell Biology, doi:10.1089/dna.2008.0735, v27(8), pp433-441.

[25] Nafasi, Sh., Manouchehri, F., Tajmir-Riahi, H.A., and Varavipour, M., 2008, Structural features of DNA interaction with caffine and theophylline, J Mol Structure, v875, pp392-399.

[26] Shohreh Nafisi, Ali Akbar Saboury, Nahid Keramat, Jean-Francois Neault and Heidar-Ali Tajmir-Riahi, 2007, Stability and structural features of DNA intercalation with ethidium bromide, acridine orange and methylene blue, J Mol Structure, doi 10.1016/j.molstruc.2006.05.004, v827, pp35-43.

[27] Taillandier, E., Liquier, J., Taboury, J. A., 1985, Infrared spectral studies of DNA conformations, Advances in infrared and Raman spectroscopy, ISSN 0309-426X, v12, pp65-114.

[28] Pupples, G. J., Otto, J. Greve, M. Robert Nicuod, D.J. Arndt Jovin, and T.M. Jovin., 1994. Raman microspectroscopic study of low-pH-induced changes in DNA structure of polyamine chromosomes, Biochemistry, v33, pp3386-339529.

[29] Domenick E. DiRico, Jr., Patrick B. Keller and Karl A. Hartman, 1985, The infrared spectrum and structure of the type I complex of silver and DNA, Nucleic Acids Res., v13, pp251-260.