*Asian Journal of Chemistry* Vol. 21, No. 6 (2009), 4237-4240

# Curcumin-Nucleotide Interaction by FT-Raman Spectroscopy

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The interaction of deoxyguanosine-5′-monophosphate (dGMP) with curcumin is investigated in aqueous solution at physiological pH with drug/dGMP (phosphate) molar ratios of r = 1/50, 1/20, 1/10 and 1/5. Fourier Transform Raman (FT Raman) spectroscopy is used to determine drug binding sites, dGMP secondary structure, as well as the structural variations of curcumin-dGMP complexes in aqueous solution. Spectroscopic evidence showed that at low curcumin concentration (1/50), drug-dGMP interaction is mainly through the backbone $PO_2$ groups. Such interaction largely perturbs the phosphate vibration near 1053 $cm^{-1}$. At higher drug concentration (r >1/10) the participation of guanine base in drug-dGMP complexation was evidenced by strong perturbations of guanine vibrations near 1717 $cm^{-1}$.

**Key Words: Curcumin-nucleotide interaction, FT-Raman spectroscopy.**

## INTRODUCTION

A major challenge in cancer therapy is the identification of drugs that kill tumour cells while preserving normal cells. On the other hand improving the dietary function has been shown to be an important determinant of human cancer risk. Several natural chemical constitutions in fruits and vegetables have now been purified and shown to protect against carcinogenesis in experimental animals and some of these compounds are now in clinical trials[1,2].

Recently, oncologists have considered curcumin as a potential third generation anticancer agent. The ongoing clinical and laboratory research indicate that dietary effect of curcuminoids stabilize and protect biomolecules in the body at the molecular level.

This stabilizing effect can be illustrated by the antioxidant anti-mutagenic and anticarcinogenic action of curcuminoids both under *in vitro* and *in vivo* conditions.

In a comparative study on the protective effects of curcumin and aspirin in mice subjected to induced thrombine challenge, curcumin was found to exhibit dose related antithrombotic effect[3]. Curcumin, like aspirin, was found to inhibit cyclooxygenase activity of platelets and platelet thromboxane $B_2$ ($TxB_2$) levels.

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However, curcumin did not affect vascular prostacyclin ($PGl_2$) synthesis. The protective effect by curcumin was directly proportional to the dose up to a level of 200 mg/kg as an intraperitoneal administration, whereas the same was inversely proportional in the case of aspiral administration.

Curcumin has the ability to protect lipids, hemoglobin and DNA against oxidative degradation. Pure curcumin has more potent superoxide anion scavenging activity than other derivatives[4]. The interaction of curcumin with polynucleotides, DNA or DNA adducts is a major biochemical importance. In the present report, the interaction of dGMP with curcumin at physiological pH with drug/dGMP molar ratios r = 1/50, 1/20, 1/10 and 1, using FT-Raman spectroscopic technique is studied that have not been previously reported.

## EXPERIMENTAL

Deoxyguanosine-5′-monophosphate (dGMP) and curcumin were purchased from the Sigma Chemicals Co. and used as supplied. A solution of dGMP (0.1 M) was prepared and kept at 4 °C for 24 h to ensure the formation of a homogeneous solution. This solution was then mixed with a solution of the curcumin to give mixture with desired drug/dGMP ratio (1:50, 1:20; 1:10 and 1:5). An aliquot of 50 µL was sealed in glass capillary tubes for Raman analysis.

FT-Raman spectra were recorded by using IFS FT-IR spectrometer coupled to a FRA 106 FT-Raman accessory (Brucker). Raman scattering was excited by a 1064 nm continuous-wave Nd: YAG laser using approximately 180 mW of radiant power at the sample. The spectra were typically recorded at a 4 $cm^{-1}$ slit width with 1000 scans. The FT-Raman accessory, used with a near-IR beam splitter collected stokes data over a range of 1800-600 $cm^{-1}$. Data collection and processing were carried out with data system CS-42 (Brucker).

## RESULTS AND DISCUSSION

**Drug: dGMP complexes**: At low drug concentration r = 1/50, no major Raman spectral changes are observed for dGMP on drug complexation. An increase in intensity is observed for several dGMP in-plane vibrations at 1709, 1674, 1622, 1592, 1488, 1383 $cm^{-1}$ (r = 1/50)[5-8]. The calculated intensity ratios of these vibrations as a function of drug concentration are shown in Fig. 1. Similarly, the band at 1709 $cm^{-1}$ due to carbonyl vibration shifts towards a lower frequency at 1701 $cm^{-1}$, while the $PO^{2-}$ symmetric stretching mode at 1079 $cm^{-1}$ exhibits intensity decrease on drug interaction. The observed spectral changes are due to an indirect drug interaction (*via* $H_2O$) with the backbone $PO^{2-}$ group[9,10].

The base unstacking also occurs with major intensity increase of the guanine vibrations at 1488, 1526 and 1622 $cm^{-1}$. It is important to note that similar increase in intensity was observed for several dG vibrations in the Raman spectra of Calfthymus DNA during G-C disruption and base unstacking upon thermal denaturation[11].

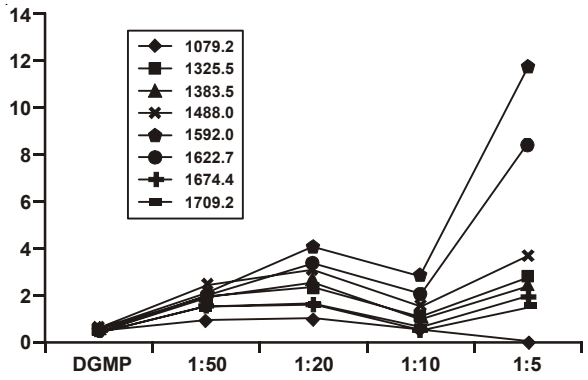


Fig. 1. Intensity ratio calculated for several dGMP in-plane vibrations at 1079.2 $cm^{-1}$ ($PO_2$ stretch), 1325.5 $cm^{-1}$ (dG), 1383.5 $cm^{-1}$ (dG), 1488 $cm^{-1}$ (dG, N7, C8 stretching), 1592 $cm^{-1}$ (dG), 1622.7 $cm^{-1}$ (dG), 1674.4 $cm^{-1}$ (dG) and 1709.2 $cm^{-1}$ (dG) as a function of drug concentration (Curcumin/dGMP molar ratios)

At r = 1/20, a minor increase in intensity is observed for dGMP in-plane vibrations at 1220, 1242, 1325, 1342, 1488, 1526.9 $cm^{-1}$. It is important to note that the intensity variations observed for the dG in-plane vibrations are accompanied by the shift of the bands at 1325 to 1328.6 $cm^{-1}$, 1342.2 to 1350.8 $cm^{-1}$ and 1488 to 1491 $cm^{-1}$. This indicates the beginning of the interaction of the drug with guanine base[12].

At r >1/20, curcumin interaction with guanine bases occurs. Evidence for this comes from a decrease in the intensity of the band at 1709 $cm^{-1}$. Similar decrease in intensity is also observed for the $PO^{2-}$ band at 1079 $cm^{-1}$. The intensity decrease of these vibrations also associated with the shift of the bands at 1709 to 1701 $cm^{-1}$ and 1709 to 1072 $cm^{-1}$. The observed changes are due to a major drug interaction with the phosphate group and the guanine bases (N-7 site). Evidence for the guanine participation in drug interaction comes also from the shift of the guanine lines 1488 and 1592 $cm^{-1}$ towards to higher frequencies, in the Raman spectra of the curcumin: dGMP complexes formed at r > 1/20. However, this type of complexation with the guanine bases does not cause nucleotide destabilization, since no major increase of intensity is observed for the dGMP in-plane vibrations.

At r > 1/10, drug-dGMP interaction continues through N-7 site of guanine bases as well as the backbone $PO^{2-}$ groups. Evidence for this comes from an intensity decrease and lower frequency shift of the Raman bands[12] at 1709 to 1696 $cm^{-1}$ and 1079 to 1066 $cm^{-1}$.

The major spectral shiftings observed for the curcumin in-plane vibrations are also characteristics of drug interaction *via* anion specific donor sites. Raman bands at 1642.92, 1610.18, 1547.85, 1461.22, 1279.95 related to C=O, C=C, C-O, C-C and $OCH_3$ vibrations, respectively exhibited major shiftings in the spectra of drug-dGMP complexes. The observed Raman spectral differences are also characteristics of curcumin-dGMP complexation through drug C=O and C-O donor atoms[13].

TABLE-1

| Frequency ($cm^{-1}$) | | | | | | Assignments |
|---|---|---|---|---|---|---|
| Curcumin | dGMP | Cur:dGMP 1:50 | Cur:dGMP 1:20 | Cur:dGMP 1:10 | Cur:dGMP 1:5 | |
| 1060.24 | 1079.2 (5.9) | 1078.0 (3.97) | 1077.0 (2.64) | 1072.5 (2.3) | 1066.04 (1.05) | Ring vibration, $PO_2^{2-}$, stretching vibration |
| – | 1220.5 (4.51) | 1230.0 (6.23) | 1219.0 (4.64) | 1223.9 (3.75) | – | $PO^{2-}$ anti-symmetric stretching vibration |
| 1252.54 | 1242.9 (4.15) | 1241.0 (6.78) | 1241.6 (5.16) | 1230.0 (3.45) | – | In-plane CH deformation, dG (in-plane ring stretching). |
| – | 1325.5 (4.36) | 1329.9 (8.01) | 1328.6 (5.86) | 1330 (3.88) | 1323.2 (2.7) | dG (in-plane ring stretching). |
| – | 1383.6 (4.72) | 1385.0 (9.59) | 1392.4 (6.32) | 1391.1 (3.88) | 1382.5 (1.97) | dG (in-plane ring stretching). |
| 1461.2 | 1488.0 (6.89) | 1499 (9.86) | 1491 (7.55) | 1500.4 (5.31) | 1489 (3.16) | $OCH_3$ deformation, dG (N7-C8 stretching). |
| 1547.85 | 1526.9 (5.7) | 1516 (9.46) | 1527 (6.77) | 1524 (4.75) | 1536.6 (2.63) | C=C stretching vibration, dG (in-plane ring stretching). |
| – | 1592 (6.26) | 1611.9 (8.6) | 1606.4 (10.00) | 1606.5 (10.0) | 1607 (10.00) | dG (in-plane ring stretching). |
| 1610.18 | 1622.7 (5.56) | 1645.5 (8.56) | 1635.3 (8.19) | 1637.0 (7.38) | 1634.4 (7.17) | C=C stretching, dG (NH bending vibration). |
| 1642.92 | 1674.4 (5.77) | 1687 (6.64) | 1685 (4.0) | 1674 (2.63) | 1696.6 (1.64) | DG (C=O stretching, $NH_2$, NH bending) vibration; C=O stretching. |
| – | 1709.2 (5.77) | 1701 (6.1) | 1701.9 (4.06) | 1708 (2.13) | 1710 (1.32) | dG $C_6$=O stretching, NH bending vibration. |

## Conclusion

The spectroscopic results presented here for the first time, clearly show that at low drug concentration (r =1/50), curcumin anion binding is mainly through the backbone $PO^{2-}$ groups. As drug concentration increases (r > 1/20), curcumin binding extends to guanine bases (N-7 site). This type of complexation does not cause guanine nucleotide perturbation.

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(*Received*: 25 March 2008; *Accepted*: 6 March 2009) AJC-7318