Structure-spectrum correlations in nucleic acids. I. Raman lines in the 600–700 cm\(^{-1}\) range of guanosine residue

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ABSTRACT

Raman spectra of nine crystals of known structures which involve guanosine moieties with various conformations have been observed. It has been established that a guanosine residue with the C3'-endo-anti conformation gives a strong Raman line at 666 ± 2 cm\(^{-1}\). It has also been found that the residue with 04'-endo-anti gives a strong Raman line at 682 cm\(^{-1}\), and C3'exo-syn at 616 cm\(^{-1}\). The usefulness of these structure-spectrum correlations in the conformation studies of polynucleotides are shown.

INTRODUCTION

Raman spectroscopy can serve as an excellent mediator between the detailed molecular conformations of nucleic acids in crystals and those in solutions (or in any other states of biological significance).

In the ordinary frequency range (200–2000 cm\(^{-1}\)), a nucleic acid gives a number of Raman lines whose positions and intensities form a rich source of information. Such a spectral feature is determined primarily by the molecular conformation of each conformer involved. It is true that crystalline force field and other environmental perturbation often cause appreciable effects on the vibrational spectrum, not only through geometrical changes, but also through potential-energy changes. As will be shown below (and in a series of our coming papers), however, the latter factors are mostly smaller than the former factors of the effects. In other words, a given conformation of a nucleic acid is considered to be correlated approximately with a unique Raman spectrum that is distinguishable from the spectrum of any other conformation. The conformation means here a local ones, such as whether a particular ribose ring has the C2'endo form or the C3'endo form, whether a guanine moiety is located at the anti position with respect to the ribose moiety through the glycosidic bond or at the syn position, and whether an O-C5'-C4'-C3' system has the trans form in the internal rotation around the C5'-C4' bond or the gauche form.
The correlation of such a conformation with the Raman spectrum is not simple; it is by no means irregular but regularity is not easily derived \textit{a priori} in our present stage of knowledge. This is a disadvantage of the Raman spectroscopy or vibrational spectroscopy in general. One of its advantages, on the other hand, is that the sample of Raman spectroscopy can be either crystal or aqueous solution (or even an intact cell, probably).

In view of such a situation, an obviously sharp tactics is to collect Raman spectra of nucleic-acid crystals, in which the molecular conformations are established by X-ray crystallography. We adopted this tactics some years ago, and have so far examined 23 mononucleotide or nucleoside crystals. These involve ten guanosine (four C3'endo-anti, one C2'endo-anti, one O4'endo-anti, one C1'exo-anti, one C2'endo-high-anti, one C4'exo-syn, and one C3'exo-syn) moieties, six cytidine (three C3'endo-anti, one C2'endo-anti, one C3'exo-anti, and one C1'exo-anti) moieties, four thymidine (one C4'exo-anti, two C2'endo-anti, and one C3'exo-anti) moieties, and five adenosine (one C3'endo-anti, one O4'endo-anti, one C1'exo-anti, one C2'endo-anti, and one C3'exo-anti) moieties. In this paper, and in our subsequent papers, we present the conformation-spectrum relations, which are extracted from the comparisons of these Raman spectra, and which are useful in the structure studies of polynucleotides in solutions, in a systematic manner.

**EXPERIMENTAL**

**Crystals**

9-\(\beta\)-5'-Phospho-D-ribofuranosylguanine (5'-GMP) trihydrate crystal was kindly provided by Mr. N. Nagashima, Central Research Laboratories, Ajinomoto Company, Inc. This was identified as what was subjected to crystallographic studies\textsuperscript{1,2}. A crystalline sample of 5'-GMP.Cd.8H\textsubscript{2}O was a kind gift of Dr. K. Aoki, the Institute of Physical and Chemical Research, and its crystal structure was determined by himself.\textsuperscript{3} 5'GMP.Ni.8H\textsubscript{2}O and 5'dGMP.Ni.8H\textsubscript{2}O crystals (where dGMP is 9-\(\beta\)-5'-phospho-2'-deoxy-D-ribofuranosylguanine were prepared by the procedures given by Gellert et al.\textsuperscript{4}, and then newly subjected to our own crystallographic study. The results confirmed that the guanosine moieties have the C3'endo-anti conformations in both of these crystals. Crystalline samples of 5'-dGMP.Na\textsubscript{2}.4H\textsubscript{2}O \textsuperscript{5} and 3',5'-cGMP.Na.4H\textsubscript{2}O \textsuperscript{6} (where 3',5'-cGMP is
guanosine-3',5'-cyclic-phosphoric acid) were purchased from Boehringer Manheim, and that of 5'GMP.Na₂.7H₂O was a kind gift of Mr. N. Nagashima. Guanosine dihydrate crystal was obtained from Wako Pure Chemicals. The X-ray powder diffractions were examined of all of these four, and they were found to be the same with what are reported in the literature. 8) A crystallographic study has newly been carried out of deoxyguanosine-dimethyl formamide complex, and C3'exo-syn guanosine has been found here. The same crystal was subjected to our Raman spectroscopic study.

Raman spectroscopic measurement

Each of the crystalline-powder samples was placed in a capillary tube, and 514.5 nm beam from an NEC GLG3300 argon ion laser was focused on it for exciting Raman scattering. The scattered light was examined by a JASCO R-800 Raman spectrophotometer with a controller and a microprocessor, and the data was stored in a 8 KW buffer memory. The data acquisition was made at 5 points per 1 cm⁻¹, and with the rate of 2 sec/cm⁻¹ in the 150-1800 cm⁻¹ spectral range. Some of the data were filed in a hard disk RLO2 through a mini-computer DECLAB 11/23 in the form of serial line. The calibration of the wavenumber scale was made by the use of the standard lines of indene. 10)

RESULTS AND DISCUSSIONS

General

The observed Raman spectra are reproduced for the 550-750 cm⁻¹ range in Figure 1. Frequencies of the Raman lines observed in the 600-700 cm⁻¹ range are listed in Table 1, with their intensities (relative intensities on assuming that the intensity of the 1575 cm⁻¹ line is 10 in each Raman spectrum).

As is seen in Figure 1,a, guanine itself gives only one Raman line in the 600-700 cm⁻¹ range, which is strong, at 650 cm⁻¹, and which is assignable to its ring breathing vibration, on the basis of a normal coordinate treatment with a set of force constants derived from an ab initio MO calculation 11). Deoxyribose, on the other hand, gives no strong Raman lines here, but it is probably important that it shows three weak Raman lines, at 685, 656, and 630 cm⁻¹ (see Figure 1, k). In other words, ribose ring has a few intrinsic vibrations in this frequency range. When guanine and ribose are linked to form guanosine, one or two strong Raman lines appear at appreciably higher or lower frequencies than 650 cm⁻¹ (Figure 1, b-j). It is natural to
consider that the intensities of these Raman lines come from the guanine ring-breathing vibration, but their positions are shifted from 650 cm\(^{-1}\) by the ribose-guanine vibrational couplings. The mode of such a vibrational coupling should depend upon the ribose conformation as well as upon the manner of guanine ribose linkage, so that the strong Raman lines in the 600-700 cm\(^{-1}\) range may be useful to diagnose the guanosine conformation.

666 cm\(^{-1}\) versus C3'endo-anti

This correlation has been well established in the present study. Thus, all of the four crystals involving the C3'endo-anti guanosine moiety give a strong Raman line at 666 ± 2 cm\(^{-1}\) (Figure 1, b-e), and the other five crystals which do not involve this conformation show no strong Raman line here (Figure 1, f-j). The applicability of this correlation seems to be expandable to the oligo- and poly-nucleotides. The Ca-salt crystal of GpC is known by an X-ray crystallographic study (12, 13) to involve the C3'endo-anti guanosine. Also, double-helical
Table 1.

Stronger Raman lines observed in the 600-700 cm\(^{-1}\) range of the crystals involving guanosine moiety

| Crystal       | Reference | Guanosine conformation | Raman line Freq. (Int.)\(^{a}\) | Curve in Fig.1 |
|---------------|-----------|------------------------|---------------------------------|----------------|
| 5'GMP.3H\(_2\)O | 1, 2)     | C3'endo-anti            | 668 (15)                        | b              |
| 5'GMP.Cd.8H\(_2\)O | 3)        | C3'endo-anti            | 668 (15)                        | c              |
| 5'GMP.Ni.8H\(_2\)O | 4, 9)     | C3'endo-anti            | 666 (16)                        | d              |
| 5'dGMP.Ni.8H\(_2\)O | 4, 9)     | C3'endo-anti            | 666 (16)                        | e              |
| 5'dGMP.Na\(_2\).4H\(_2\)O | 5)        | O4'endo-anti            | 694 (6)                         | f              |
| guanosine.2H\(_2\)O | 8)        | C1'exo-anti             | 673 (13)                        |                |
|                |           | C2'endo-high-anti       | 665 (-6)                        |                |
| 5'GMP.Na\(_2\).7H\(_2\)O | 7)        | C2'endo-anti            | 676 (8)                         | h              |
| 3'5'cGMP.Na.4H\(_2\)O | 6)        | C4'exo-syn              | 674 (7)                         |                |
| deoxyguanosine.DMF | 9)        | C3'exo-syn              | 680 (13)                        |                |
|                |           |                        | 617 (12)                        |                |

\(^{a}\) Relative intensity on assuming that 1575 cm\(^{-1}\) line intensity = 10.

RNA as well as the A-form DNA are known to have the C3'endo-anti guanosine. All of these give a strong Raman line in the range of 665-668 cm\(^{-1}\).

It is interesting that this correlation seems to be valid even when the guanine ring is protonated at N7, as is the case for 5'GMP.3H\(_2\)O (Figure 1,b). In addition this correlation is good both for guanosine and deoxyguanosine (see, for example, Figure 1, d and e). In detail, however, the marker line frequency seems to be slightly higher for guanosine than that for deoxyguanosine. Thus, it is found at 666 cm\(^{-1}\) for 5'GMP.Ni.7H\(_2\)O and at 664 cm\(^{-1}\) for 5'dGMP.Ni.7H\(_2\)O (These two have very similar crystal structures to each other). Also, double-helical RNA gives a Raman line at 668 cm\(^{-1}\), whereas A-form DNA at 665 cm\(^{-1}\).

680 cm\(^{-1}\) versus C2'endo-, C1'exo-, or O4'endo-anti

As is seen in Figure 1, f-h, all the guanosines with anti glycosidic linkage and with a ribose puckering other than C3'endo give a strong Raman line at a higher frequency (in the 673-682 cm\(^{-1}\) range) than
Enlarged reproductions of the Raman spectra (f), (g), and (h) in Figure 1 for 655-705 cm\(^{-1}\) spectral region.

666 cm\(^{-1}\). It is evident, in addition, that a further detail of the conformation is reflected in the Raman frequency here, with apparent correlations of 676 cm\(^{-1}\) versus C2'endo-anti, and 682 cm\(^{-1}\) versus O4'-endo-anti (see Figure 2). The guanosine-dihydrate crystal involves two different conformations of the guanosine molecule C1'exo-anti and C2'endo-high anti (where "high anti" means that O4'-C1'-N9-C4 dihedral angle is as great as 300° instead of 220° for usual anti). This gives a broad and strong Raman band at 673 cm\(^{-1}\) (Figure 2), and its interpretation is not simply made. It is helpful, however, to know that an acidic gel of 5'-GMP (at pH 4.5), which is considered to involve C1'exo-anti guanosine (as will be detailed elsewhere), gives a strong Raman line at 673 cm\(^{-1}\). Thus a correlation of 673 cm\(^{-1}\) versus C1'exo-anti is suggested. We should increase our crystallographic collection before we establish the structure-spectrum correlation in the 670-680 cm\(^{-1}\) region.

The so-called B-form DNA shows always a strong Raman band at 682 cm\(^{-1}\). On the basis of our present finding just described, it is
Figure 3. Raman spectra of two crystalline-powder samples of mononucleotide and an aqueous solution of dG-dC alternating copolymer in the 1300-1400 cm\(^{-1}\) region.

suggested that the B-form DNA involves an O4'endo-anti guanosine rather than a C2'endo-anti which is usually postulated. This suggestion is supported by our Raman spectroscopic examination in the 1300-1400 cm\(^{-1}\) range. As is seen in Figure 3, O4'endo-anti guanosine and C2'endo-anti guanosine give distinguishably different Raman spectra from each other in the 1300-1400 cm\(^{-1}\) region. So-called B-form of poly[d(G-C)\_poly[d(G-C)]] (in low-salt aqueous solution) gives a Raman spectrum similar to that of O4'endo-anti guanosine rather than to that of C2'endo-anti guanosine. The structure-spectrum correlations of guanosine in the 1300-1400 cm\(^{-1}\) region will be detailed in our coming paper.

620 cm\(^{-1}\) versus syn

As is illustrated by two examples (Figure 1, i and j), the characteristic of the syn-guanosine is a strong Raman line at a lower frequency range, 620-630 cm\(^{-1}\). It has now been established that the characteristic Raman line at 625 cm\(^{-1}\) of Z-form DNA\(^\text{15}\) comes from the syn form guanosine, rather than from the left-handedness itself. In using this correlation, however, one must be careful of the following two points: (i) the 620 cm\(^{-1}\) line is more or less accompanied by another Raman line at about 680 cm\(^{-1}\), and (ii) the relative intensities of these two Raman lines as well as their positions depend upon the ribose conformation. The intensity ratio of 620/680 cm\(^{-1}\) is about 1/1 for C3'exo-syn or for C4'exo-syn (see Figure 1, i and j) while it is considered to be as great as 4/1 (or even more) for C3'endo-syn.
The d(CGCGCG) crystal is known to involve two C3'endo-syn and one C2'endo-syn (or C3'exo-syn) guanosines. On the basis of the correlation postulated above, this should give two Raman lines at 620 and 680 cm\(^{-1}\) with a ratio of \((4/5 + 4/5 + 1/2):(1/5 + 1/5 + 1/2) = 0.4\). This is what actually observed. Poly[d(G-C)].poly[d(G-C)] in high-salt medium shows strong 625 cm\(^{-1}\) Raman line and very weak scattering in the 680 cm\(^{-1}\) region. This probably means that it involves only C3'endo-syn guanosine.

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