Vol 16 No 11, November 2007
1009-1963/2007/16(11)/3332-07

Chinese Physics

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Raman spectroscopic study of G–A mismatches*

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(Received 21 December 2006; revised manuscript received 21 March 2007)

G–A mismatches are non-canonical base pairs that widely occur in native nucleic acids. They have been found to be functionally important in adopting unusual structures. In this paper, G–A mispairing was studied by Raman spectral characterization of Polyadenylic acid (PolyA), Polyguanylic acid (PolyG) and their equimolar mixture in solution of 0.08 mol/L Na^+, pH7.0. The experiment showed the following three results. (1) At the experimental conditions used in the present work, PolyA and G–A complexes existed as single-stranded and double-stranded helix of A form, respectively. And PolyG mainly occurred to be anti-parallel quadruplex conformers. (2) The formation of G–A complexes, on one hand strengthened parts of base stacking interactions especially for PolyG, leading to Raman hypochromism effect with some corresponding bands shifting, and on the other hand weakened other base stacking interactions especially for PolyA to a certain degree. During this process, the backbone of PolyG underwent a significant change, but PolyA still conserved its main chain conformation. (3) The formation of G–A complexes was stabilized by two interbase hydrogen-bond interactions (i.e. $N6H_2$(A)—N3 (G) and N7 (A)—$N2H_2$(G)) and a third hydrogen bond between O2' (G) and N6 (A). The third hydrogen bond was responsible for the remarkable changes of PolyG backbone conformation.

Keywords: G–A mismatches, Raman spectroscopy, Polyadenylic acid (PolyA), Polyguanylic acid (PolyG)

PACC: 3320F, 8715B, 3620E

1. Introduction

Recently, non-canonical base pairs, such as G–A, A–A, G–T mismatches etc, have attracted much attentions[1−5] due to their thermodynamic stability, widespread occurrence and polymorphism.[6−8] These so-called mismatches in DNA or RNA are generally found at biologically significant locations.[9−15] They occur naturally as a result of errors during DNA replication and recombination or as a consequence of mutagenic chemicals or ionizing radiation, which can give rise to conformation anomalies. Repair of these mismatches requires their recognition by proofreading enzymes or by postreplication repair systems.[3] So, accurate knowledge of interactions between noncomplementary bases and structures of those mispairs will help in our understanding of these repair processes.

Raman spectroscopy has been proved valuable for structural analysis of a variety of materials.[16−23] And it is especially a powerful tool for study of biological materials, such as nucleic acids, proteins etc. With this technique, unique information on hydrogen bonding, local conformation, and base stacking of nucleic acids[24−29] can be obtained. This work presents the first Raman results for G–A mismatches by using the model compounds PolyG and PolyA. There are two reasons to choose this system. First, the study of synthetic polynucleotides has been proved to be one of the most fruitful approaches to understanding the natural nucleic acids as well as provided detailed and fundamental chemical information of wide general interest.[30−38] Second, polynucleotides consist of a linear array of only one nucleotide residue. Due to uniform primary structures, they are quite suitable for spectral analysis of the transition behaviour of ordered polymers as a result of mispairing.[39] Our aim in this study is to confirm the sensitivity of Raman spectra to structural deviations of mismatched duplexes and obtain characteristic spectral signatures on the special complexes.

*Project supported by the Natural Science Foundation of Guangdong Province, China (Grant No 021093) and Key-stone Science and Technology Foundation of Guangdong Province, China (Grant No 2KM04502S).
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2. Materials and methods

2.1. Chemicals and polynucleotides

PolyG and PolyA were synthesized by Sigma-Aldrich and Pharmacia-Biotech respectively, and were used without further purification. Anhydrous sodium sulfate solid in analytical grade was dissolved in three-distilled water to 0.04 M. PolyG and PolyA were run as 5% (by weight) solutions in 0.04 M sodium sulfate respectively. And the concentrations of the two samples were both 0.165 M in nucleotides. The mismatched duplex (PolyG–PolyA) was prepared by mixing equimolar PolyG and PolyA strands in 0.04 M sodium sulfate solution, and the concentration of either strand was the same as that in the single strand solution. The pH of these solutions, measured with a microelectrode and an Orion Model 721 pH meter, was adjusted to 7.0 with HCl or NaOH. All the solutions were stored at 4°C for about one week until recording the spectra.

2.2. Raman spectroscopy

The Raman spectra of the samples contained in capillary tubes were excited by the 514.5-nm line of an argon ion laser (Coherent Co.) (about 5.8 mW of laser power on the sample) and recorded on a Renishaw RM2000 system equipped with a CCD detector (at an ambient temperature of 25°C). The effective spectral resolution was about 1–2 cm^{-1}. The reported Raman frequencies were calibrated by using the 520 cm^{-1} Raman band of Silicon. The peak wavenumber values of Raman bands were reproducible within ± 0.05 cm^{-1}. Three scans were accumulated for each spectrum, and the laser exposure for each scan was 30 seconds. No sample degradation was observed both visually and spectrally during measurements. Spectral data treatment was performed by using Origin 6.0 software. And all the Raman spectra were normalized to the intense SO_4^{2-} band at 981 cm^{-1}.

3. Results and discussion

3.1. Results

Raw Raman spectra of PolyA, PolyG, G–A complexes as well as solvent background are shown in Fig.1(a). Figure 1(b) presents the corresponding results which have been baseline-corrected and smoothed. For clarity, the spectra have been offset on the Y-axis.

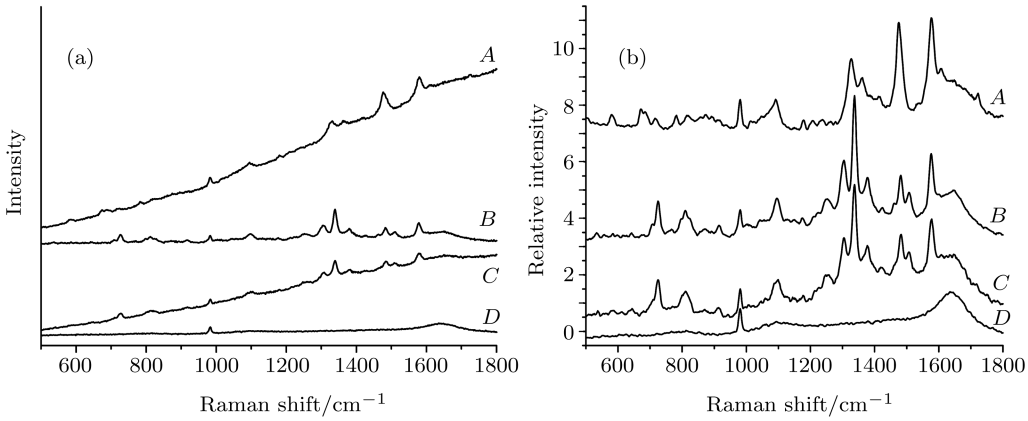


Fig.1. (a) Raw Raman spectra of PolyG (A), PolyA (B), G–A complexes (C) as well as aqueous solution background (D) and Fig.1(b) the corresponding results which have been baseline-corrected and smoothed. Conditions: 0.04 M sodium sulfate solution with pH value of 7.0 at 25 °C.

3.1.1. Base stacking and interbase hydrogen bonds

Bands appearing between 1200 cm^{-1} and 1800 cm^{-1}, combining with the spectral region between 500 cm^{-1} and 750 cm^{-1}, mainly reflect base stacking and interbase hydrogen bonds, with the exception of those measured between 1400 cm^{-1} and 1470 cm^{-1}, which correspond to methylene and methyl bending vibration.

In the double-bond region (1600–1750 cm^{-1}) (Fig.2(a)), two Raman bands of PolyG are detected, with frequencies at 1609 cm^{-1} and 1723 cm^{-1} respec-

tively. The former is attributed to NH_2 scissoring of guanine ring [40,41] and the latter originates from carbonyl stretching.[42–44] In Fig.2(a)–curve *A*, both of the two bands can be seen clearly. While in Fig.2(a)–curve *C*, they both decline in intensity. Besides, the band 1609 cm^{-1} changes its frequency centre from 1609 to 1607 cm^{-1}. Since the 1609 cm^{-1} mode is sensitive to NH_2 hydrogen-bond donor sites,[45,46] its intensity change combined with the band shift reflects the alteration of interbase hydrogen bonds. The band 1723 cm^{-1} has been assigned to C=O of self-associated PolyG in quadruplex form.[44–47] Therefore a great loss in intensity may indicate breakage of quadruplex hydrogen bonds upon mismatching.

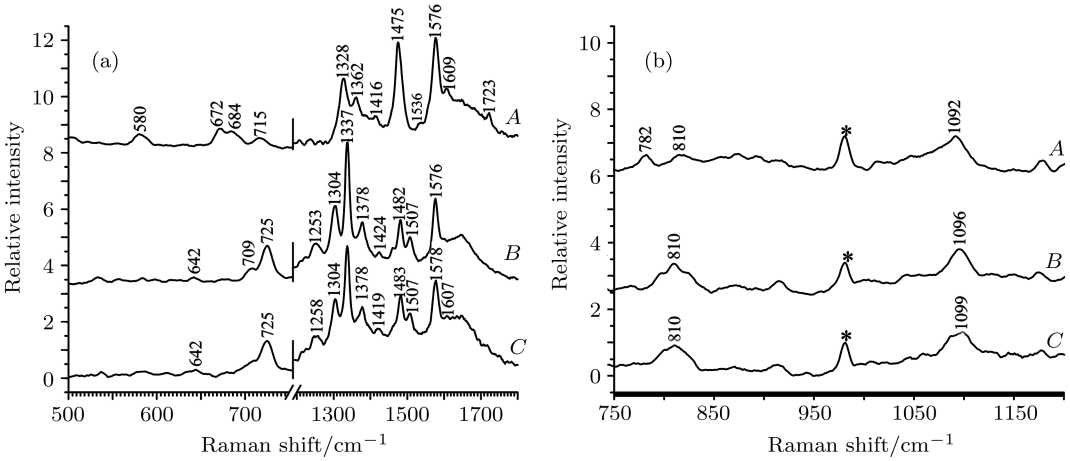


Fig.2. Raman spectra in detail of PolyG (*A*), PolyA (*B*) and G–A complexes (*C*). All the spectra were normalized to the intense SO_4^{2-} band at 981 cm^{-1} (designated by asterisk). The conditions were the same as for Fig.1.

Bands in the 1200–1600 cm^{-1} region are sensitive indicators of ring electronic structures. They are expected to exhibit perturbations upon mismatching. Unfortunately, the overlapping modes of adenine and guanine make the characterization of band changes separately somewhat difficult, especially for those bands of PolyG. Thus little can be said about the change of the two PolyG bands at 1328 cm^{-1} and 1362 cm^{-1}. But from the figure we can still observe that some other Raman bands have remarkably changed. First, two strong bands of PolyG modes and those of PolyA modes peaking at 1475 cm^{-1}, 1576 cm^{-1} and 1482 cm^{-1}, 1576 cm^{-1} respectively generate upshifts upon G–A interactions. Since the 1475 cm^{-1} band is sensitive to N7 hydrogen-bond interaction of guanine ring, the upshifts can be interpreted as the rupture of N7 to $N2H_2$ self-associated hydrogen bonds. This is the same behaviour as that shown for a Raman study on the melting of PolyG above 100°C, which suggested the transition of PolyG from four-strand structure to single-strand form.[45] A loss of intensity at 1536 cm^{-1} exhibited in the figure and the band shift of 1609 cm^{-1} stated above also support this assignment. When quadruplex PolyG disrupts, the N7 acceptor sites of adenine rings bond to $N2H_2$ sites of guanine. It can be speculated from the change of the 1482 cm^{-1} band of PolyA.[48,49] On the other hand, the frequency change of 1576 cm^{-1} band in PolyG modes is an indication of hydrogen-bonded N3 sites.[40,50–52] These hydrogen-bond sites are most likely to be linked to N6 amino donor sites of adenine. And the band shift of PolyA from 1576 cm^{-1} to 1578 cm^{-1} can prove it.[37,48] Second, some separated adenine bands yield intensity changes, like 1378 cm^{-1} (adenine ring stretching) increasing 18.4% and 1507 cm^{-1} (adenine ring stretching) increasing 42.9% respectively. Besides, other bands showed some small but detectable changes, like 1337 cm^{-1} decreasing 6.8% and 1253 cm^{-1}(NH_2 of adenine) increasing 8.5% with its frequency shifting to 1258 cm^{-1}. The band shift of 1253 cm^{-1} is related to that of 1576 cm^{-1} and also supports N6 amino hydrogen-bonded sites.[49,53]

As displayed in Fig.2(a), the low wavenumber region (500–750 cm^{-1}) is dominated by Raman bands assigned to the ring breathing modes of base residues with part contribution of glycosidic bond stretching to the vibration. Two medium bands of PolyG are observed at 580 cm^{-1} and 672 cm^{-1}, and the latter is a marker for the C3'-endo/anti conformation,

i.e. type A conformation. Upon PolyA addition, they both suffer a great loss in intensity, 49.5% and 66.5% respectively. This phenomenon is called Raman hypochromism and can be a strong indication of increased base stacking.[54−56] On the contrary, PolyA exhibits a weak band at 642 cm^{-1} and a strong band at 725 cm^{-1} respectively. Yet no detectable change is observed upon mismatching. Interestingly, from Fig.2(a), we can see that the band 672 cm^{-1} is actually a doublet, with a tail in the higher frequency side (about 684 cm^{-1}). So we propose that there is an appreciable amount of fluctuation of the guanosine conformation towards C2'-endo/anti conformation (type B form).[57]

3.1.2.The main chain conformations

Figure 2(b) exhibits the Raman spectra of PolyG, PolyA and G·A complexes in the region 750–1200 cm^{-1}. In this spectral region we mainly find Raman bands characteristic of the phosphate backbone geometry. As can be seen from the figure, all the three samples show a Raman band near 810 cm^{-1}. This band is assigned to 5'C-O-P-O-C3' network and arises from a specific conformation of the sugar phosphate backbone, indicating of type A RNA,[58−60] i.e., C3'-endo/anti conformers. This Raman band in the spectrum of PolyG is not as strong as that in the spectra of the other two samples. In addition, a band near 782 cm^{-1} which is assignable to 5'C-O-P-O-C3' network of B form,[61] occurs almost the same intensity as the 810 cm^{-1} band. Considering that there is no band near 835 cm^{-1}, we propose for PolyG an A/B (mainly A) type conformation. There are also some differences between the band 810 cm^{-1} of PolyA and that of G·A complexes. The former polymer has a shoulder located at about 795 cm^{-1}, which is diagnostic of some disordered content.[62] This indicates that unlike double-stranded helix G–A complex, PolyA shows more flexibility in main chain structure. In neutral pH aqueous solution, it occurs as single-stranded helix due to strong stacking interaction between adenine bases.[63] Interestingly, upon PolyG addition, the shoulder at 795 cm^{-1} emerges, and the band 782 cm^{-1} of PolyG also disappears. These changes suggest the alteration of main chain structures.

The strong band near 1100 cm^{-1} is due to the symmetric stretching vibration of the PO_2^- moiety.[61,64,65] Unlike the band at 810 cm^{-1}, the intensity of this band is not very sensitive to backbone conformations. The bands are located at 1092 cm^{-1}, 1096 $cm^{-1}$and 1099 cm^{-1} for PolyG, PolyA and G–A complexes respectively. It is unexpected that the band for PolyG appeared at relatively lower frequency, because in this region we usually find nucleic acids of B family.[56,65] And we are also surprised to see that the 1099 cm^{-1} band of G·A complexes includes actually two bands with the higher frequency at 1099 cm^{-1} and the lower one at 1087 cm^{-1}. Furthermore, we find that the FWHM (the full width half the maximal) of PolyA and PolyG bands are the same (39 cm^{-1}), while the band of G–A complexes shows remarkable broadening (61 cm^{-1}).

Thomas and other researchers[64,66] pointed out that the Raman intensity ratio $R = I_{810}/I_{1100}$ was directly proportional to the number of ordered nucleotide subgroups of A family. This has been applied not only to A-RNA duplexes and DNA (I_{806}/I_{1099}) of various types of sequences, but also to single-strand RNA. Here, the R-value for PolyG, PolyA and G–A complexes are 0.436, 0.690 and 0.689 respectively. This result, on one hand, supports the conclusion that PolyA under study contained more A form content than PolyG and on the other hand suggests rare conformational change of the main chains of PolyA upon mismatching. Let us reconsider the doublet of G–A complexes located at 1099 cm^{-1}. If we assign the band 1087 cm^{-1} of the doublet to PolyG and the band 1099 cm^{-1} to PolyA, we will have two R-values, 0.764 and 0.689, for 1087 cm^{-1} and 1099 cm^{-1} respectively. Thus, our conclusion is that mismatching improved the A form content of the PolyG strands, leading to the disappearance of the band 782 cm^{-1}, but PolyA still conserved its backbone conformation.

3.2. Discussion

3.2.1.The base pairing forms of quadruplex PolyG and G–A mismatches

As aforementioned, under present study (0.08 mol/L Na^+, pH7.0) PolyG occurs to be in the four-stranded form. To our knowledge, this special helical structure has been found in PolyI,[67] d (T_4G_4)[68] and in the melting transition of PolyG–PolyC.[45] Formation of quadruplex PolyI requires high ionic strength. So in aqueous solutions of low ionic strength, PolyI may be classified as a disordered, single-stranded polynucleotide chain. But this seems not the case for PolyG. Since the ionic strength used here is relatively lower, the presence of G quar-

tet is probably due to the high concentration of the polymers. A previous work by Pascal Mercier and coworkers[45] used much higher ionic strength. However, our spectrum of PolyG is found to be consistent with the data of their study. They both exhibit several anomalous features relative to B family. The presence of 1092 cm^{-1} and 782 cm^{-1} bands are diagnostic of type B backbone geometry. The marker bands at 672/684 cm^{-1} and 1328 cm^{-1} described above are also due to C2'-endo conformations of B family. According to Refs.[45,69], we can infer that the PolyG quartet in this study exists as anti-parallel foldback form.

The structure of self-associated PolyG was depicted in Ref.[45], in which each base in a given tetrad is linked to its neighbours by two pairs of hydrogen bonds, i.e. N1-H—O-C6 and NH_2—N7, which leaves the N3 acceptor sites exposing to solvent. From the spectral analysis that we have made, we know that it is the bonding of PolyA $N6H_2$—PolyG N3 and PolyA N7—PolyG $N2H_2$ which disturbed the pairing of quadruplex hydrogen bonds, leading to the collapse of self-associated PolyG and eventually the formation of G–A mispairs.

3.2.2.Raman difference spectra and the alteration of backbone conformations

In order to further help our study of G–A complexes, we present Raman spectra of pure PolyG, pure PolyA and pure G–A mispairs by subtracting the solvent spectrum from their raw spectra respectively. Raman difference spectrum computed by using the spectrum of pure G–A complexes as minuend and the spectral sum of components as subtrahend is also shown in Fig.3. As we can see from these 'water free' spectra, the bands such as 1609 cm^{-1} and 1723 cm^{-1} observed previously become more noticeable in the double-bond region. And the Raman difference spectrum seems to carry more information.

The most conspicuous features of the difference spectrum are those negative bands measured at 1576 $cm^{-1}(-)$, 1475 $cm^{-1}(-)$, 1336 $cm^{-1}(-)$, 1357 $cm^{-1}(-)$, 580 $cm^{-1}(-)$ and 670 $cm^{-1}(-)$ which have been described before. Considering that many of these bands can be attributed to PolyG, we find that the negative features imply strengthened base-stacking interactions of guanine in G–A mispairs. At the same time, there are also several positive peaks located at 1249(+), 1511(+) and 1527(+). The former two belong to adenine and the latter one corresponds to 1536 cm^{-1} that has been assigned to guanine N7. The presence of these positive bands reflects that some of the base-stacking interactions, especially for PolyA, were weakened by G–A mismatching.

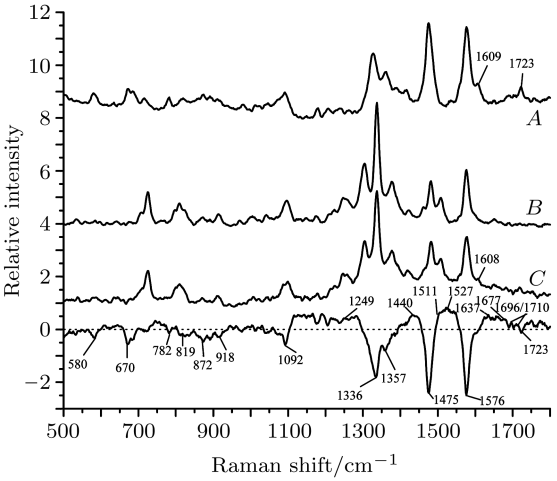


Fig.3. 'Water free' Raman spectra of PolyG (*A*), PolyA (*B*) and G–A complexes (*C*). The trace in the bottom is the Raman difference spectrum computed using the spectrum of pure G–A complexes as minuend and the spectral sum of the components as subtrahend.

In the double-bond region, we observed five bands located at 1723 $cm^{-1}(-)$, 1696 $cm^{-1}(+)$/1710 cm^{-1} $(+)$, 1637 $cm^{-1}(+)$ and 1677 $cm^{-1}(+)$ respectively. Since the band 1696 cm^{-1} is a spectral marker for free carbonyl bonds,[70,71] loss of intensity at 1723 cm^{-1} and gain at 1696 cm^{-1} strongly indicate the disruption of four-stranded PolyG. On the other hand, the band at 1710 cm^{-1}, according to its frequency, is ascribable to carbonyl motions of dimers, suggesting incomplete disruption of self-associated PolyG. The presence of 1637 $cm^{-1}(+)$ and 1677 $cm^{-1}(+)$ is due to NH_2 modes and reflects the alteration of amino hydrogen bonds.[72,73]

In addition, another interesting feature is that many bands attributed to backbone geometry also appear in the difference spectrum, such as 782 $cm^{-1}(-)$, 819 $cm^{-1}(-)$, 872 $cm^{-1}(-)$, 918 $cm^{-1}(-)$, 1092 $cm^{-1}(-)$ and 1440 $cm^{-1}(+)$. The bands at 782 $cm^{-1}(-)$ and 819 cm^{-1} $(-)$ predominantly belong to 5'C-O-P-O-C3' network of PolyG. Bands at 872 $cm^{-1}(-)$ and 918 $cm^{-1}(-)$ are assigned to ribose modes. And the band 1092 $cm^{-1}(-)$ is due to PO_2^- symmetric stretching. As for 1440 $cm^{-1}(+)$, it mainly reflects the increase of backbone CH_2 bending modes. According to Ref.[8], we propose that an extra hydrogen bond exists between O2' (G) and N6 (curve *A*). As a result, the involvement of the third hydrogen

bond combined with the other two stated above stabilizes the structure of G–A complexes on the one hand, and on the other hand leads to significant distortion of the backbone conformation of PolyG strands. This explanation is consistent with foregoing discussion on the changes of R-value.

4. Conclusions

In conclusion, we have studied G–A mispairing by Raman spectral characterization of PolyA, PolyG and their equimolar mixture in solution of 0.08 mol/L Na^+, pH 7.0. We have found that PolyA exists as single-stranded helix of A family, and PolyG mainly occurs to be anti-parallel quadruplex conformers. Upon the formation of G–A mismatches, some bands assigned to hydrogen bonding underwent small but detectable shift, which reflects the base mispairing between guanine and adenine. At the same time, significant changes in the Raman spectra of base electronic structures were observed. This indicates that base stacking interactions have been altered due to mispairing. During this process, the backbone of PolyG underwent a significant change, while PolyA still conserved its main chain conformation.

The experimental results shown above exemplify in detail the structure of G–A complex, and confirm the suitability of Raman spectroscopy to monitor conformational changes upon mispairing. Considering the sensitivity of Raman spectra to subtle conformational changes, it would be of interest to know the structures of other mispairing forms by using this technique. Further Raman studies in progress will attempt to characterize C–A, G–U and C–U mismatches. This knowledge, we believe, should then provide a foundation for the Raman studies of nucleic acid oligomers and polymers currently underway in our laboratory.

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