Characterization of PolyA and PolyC mismatches by Raman spectroscopy

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A·C mismatches are studied by Raman spectral characterization of PolyA, PolyC, and their equimolar complex in solution of 0.14 mol/L Na^+, pH7.0. Experimental results show that A·C mismatches occur to be A/B (mainly A) conformers, and unlike Watson-Crick base pairing, this kind of mismatches is stabilized by only one hydrogen bond involving cytosine $N4H_2$ and adenine N7. The formation of A·C complex makes the base stacking interactions much stronger, and conformation of the backbone more ordered, which leads to obvious Raman hypochromic effect with some shifts in corresponding bands.

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Recently, non-Watson-Crick base pairs such as G·A, A·A, G·U mismatches etc., have attracted much attention[1−5]. These so-called mismatches are often found in ribonucleic acids (RNAs), ribosomal RNA, ribozyme and viroids[6,7], and can be functionally important in adopting unusual structures. Studies on a number of RNA oligomers and ribozymes have shown that mismatches can form folded RNA structural motifs or a part of internal loop, providing recognition sites for proteins, metal ions and small molecules. To properly understand the role of non-Watson-Crick regions of RNA, it is crucial to study the structures of those mispairs and interactions between non-complementary bases. Raman spectroscopy is powerful for examining both thermodynamic and structural properties of nucleic acids in solution. With this technique, unique information on hydrogen bonding, local conformation, and base stacking of nucleic acids can be obtained[8,9]. This work presents the Raman results for A·C mismatches by using synthesized polynucleotides PolyA and PolyC. Our aim is to confirm the sensitivity of Raman spectra to structural deviations of mismatched duplexes and obtain characteristic spectral signatures on the special complexes.

The samples PolyA and PolyC were purchased from Sigma-Aldrich and Pharmacia-Biotech, respectively, and used without further purification. The used NaCl was in analytical grade and dissolved in three-distilled water to 0.06 mol/L. Anhydrous sodium sulfate solid used as an internal standard was added to NaCl solution to 0.04 mol/L. Solutions of PolyA and PolyC (both 0.165 mol/L in nucleotides) were respectively prepared by dissolving in 0.04-mol/L sodium sulfate and 0.06-mol/L NaCl solution. The mismatched duplex (PolyA-PolyC) was prepared by mixing equimolar PolyA and PolyC strands in sodium sulfate and NaCl solution, and the concentrations of both strands were the same as those in the single strand solutions. The pH value 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 before recording the spectra.

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 charge-coupled device (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 using the 520-cm^{-1} Raman band of silicon. The peak wavenumber values of Raman bands were reproducible to within ± 0.05 cm^{-1}. Three scans were accumulated for each spectrum, and the laser exposure for each scan lasted 30 s. Spectral data treatment was performed using Origin 6.0 software, and all the Raman spectra were normalized to the intense SO_4^{2-} band at 981 cm^{-1}.

Figures 1 and 2 exhibit the Raman spectra of PolyC, PolyA and the mismatched duplex PolyA-PolyC in the region $650-1150$ cm^{-1} and $1150-1800$ cm^{-1}, respectively. For clarity, the spectra have been offset on the y-axis.

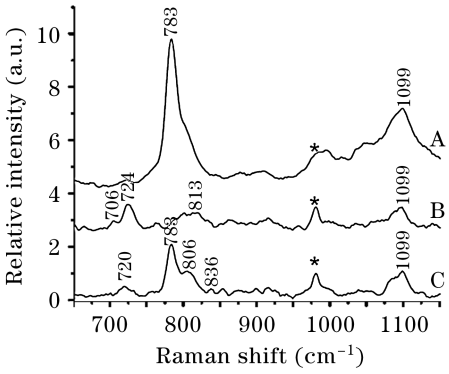

Fig. 1. Raman spectra of PolyC (A), PolyA (B) and PolyA-PolyC (C) in the region of $650-1150$ cm^{-1}. All the spectra are normalized to the intense SO_4^{2-} band at 981 cm^{-1} (designated by asterisk). Conditions: 0.06-mol/L NaCl, 0.04-mol/L sodium sulfate solution with pH value of 7.0 at 25 °C.

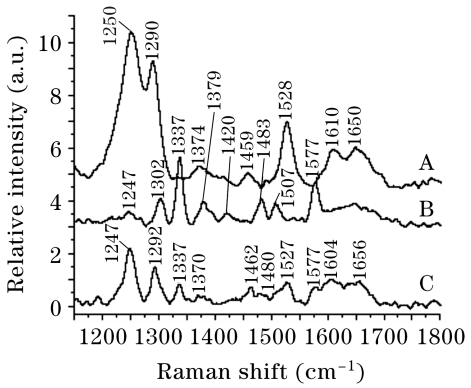

Fig. 2. Raman spectra of PolyC (A), PolyA (B) and PolyA-PolyC (C) in the region of $1150-1800$ cm^{-1}. The conditions are the same as those in Fig. 1.

The phosphate groups of A-RNA are known to give rise to two prominent Raman bands, i.e. 810 and 1099 cm^{-1} that serve as a direct evidence for A-form structure. The 810-cm^{-1} band can be assigned to 5'C-O-P-O-C3' network. Its frequency is very sensitive to subtle conformational variants. From Fig. 1, we can see the bands locating at 813 and 806 cm^{-1} respectively stand for PolyA and A·C complex. Besides, a weak band at 836 cm^{-1} which is assignable to 5'C-O-P-O-C3' network of B form appears in the A·C complex spectrum. According to Refs. [10 − 12], we can conclude that PolyA exists as single-strand A-form helix structure, and A·C complex adopts A/B (mainly A) backbone conformation. Since no counterpart is detected in the spectrum of PolyC, the conformation of PolyC is proposed to be random coiled[13].

The band near 1100 cm^{-1} is due to the symmetric stretching vibration of the PO_2^- moiety. Unlike the band 810 cm^{-1}, the frequency of this band is not very sensitive to subtle conformational modification. As can be seen from Fig. 1, although the frequency of this band is almost the same in all of the three spectra, the bandwidths are found to be different. The full-width at half-maximum (FWHM) of PolyA (30 cm^{-1}) and A·C complex (33 cm^{-1}) bands are almost the same, while that of PolyC shows remarkable broadening (65 cm^{-1}). We consider that this is probably related to the disordered chain of PolyC.

Some researchers[12,14] 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 A-DNA (I_{806}/I_{1099}) of various types of sequences, but also to single-strand RNA. Here, the R values for PolyA and A·C complex are 0.786 and 0.991, respectively, which means more ordered structure in the mismatched duplex.

In the spectral region above 1600 cm^{-1}, two Raman bands of PolyC appear with frequencies at 1610 and 1650 cm^{-1}, respectively. The former is mainly attributed to $\delta(NH_2)$, ν (C4 = N3) and ν (N1C6) of cytosine modes. The latter originates from the coupling of νC = O and νC5 = C6[15]. As shown in Fig. 2, upon the formation of mismatched duplex, both of the two bands generate shifts (−6 and +6 cm^{-1}, respectively). Obviously, these changes reflect the base mispairing between cytosine and adenine.

Bands appearing between 1200 and 1600 cm^{-1}, combining with the spectral region between 650 and 800 cm^{-1}, mainly reflect base stacking, with the exception of those measured between 1400 and 1470 cm^{-1}, which correspond to methylene and methyl bending vibrations. Upon the formation of mismatched duplex, significant spectral changes are also detected in this region. Firstly, bands due to base ring modes suffer a great loss in intensity, such as 724, 1337 and 1577 cm^{-1} of PolyA, and 783, 1290 and 1528 cm^{-1} of PolyC. This phenomenon is called Raman hypochromism and can be a strong indication of increased base stacking[16−18]. Secondly, bands such as 1250 cm^{-1} (cytosine), 1290 cm^{-1} (cytosine), 724 cm^{-1} (adenine), 1379 cm^{-1} (adenine), and 1483 cm^{-1} (adenine) show changes both in frequency and intensity. The band 1250 cm^{-1} is sensitive to $N4H_2$ hydrogen-bond site of cytosine ring[19], therefore its downshift can be interpreted as hydrogen-bonded amino. The band shift of 1610 cm^{-1} stated above also supports this explanation. Since the band 1483 cm^{-1} is a sensitive indicator of adenine N7 acceptor site[19,20], the 3-cm^{-1} downshift suggests that the N7 site is involved in the base mispairing. There is no evidence that other sites are involved in. Thus, our conclusion is that unlike Watson-Crick base pairing, the A·C complex contains only one hydrogen bond between cytosine $N4H_2$ and adenine N7.

In conclusion, under the experimental conditions used in the present work (0.06-mol/L NaCl, 0.04-mol/L Na_2SO_4 aqueous solution, neutral pH and 25 °C), A·C mismatches are found to exist as A/B (mainly A) conformers. Unlike Watson-Crick base pairing, this kind of mismatches are stabilized by only one hydrogen bond involving cytosine $N4H_2$ and adenine N7. The formation of this complex makes the base stacking interactions much stronger, and conformation of the backbone more ordered, which leads to obvious Raman hypochromic effect with some shifts in corresponding bands.

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