Raman study of magnesium induced conversion of polyU·polyA duplexes to polyU·polyA·polyU triplexes

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Abstract. Raman titration experiment with magnesium salt added gradually to aqueous solution of duplexes formed by RNA homopolynucleotides polyU and polyA was performed to reveal its effect on homopolynucleotide complexes. Statistical analysis of obtained spectral set has confirmed the effect already found by less structurally sensitive methods [Nucleic Acids Res. 31(17) (2003), 5101–5107] that at sufficiently high concentrations magnesium causes transformation of polyU·polyA duplexes to polyU·polyA·polyU triplexes and single polyA strands. It was found that at relatively high polynucleotide concentrations used in Raman experiment, the threshold magnesium concentration for this effect is above the concentration of duplex base-pairs in solution. Due to the strong spectral changes attributed to the varied percentages of duplexes, triplexes and single strands, it was not possible to register weaker direct Raman signs of the magnesium binding to polynucleotide strand.

Keywords: Duplex, triplex, polyA·polyU, polyA, polyU, homopolynucleotide, magnesium, Raman spectroscopy, RNA

1. Introduction

Nucleic acids (NA) are negatively charged poly-ions, which need extra-molecular positive charges in order to fold in different compact structures and to carry out their functions [2]. Magnesium is known as an alkaline earth metal playing an important role in stabilization of biomolecular structures including nucleic acids. It is the divalent ion with the highest intracellular concentration (concentration of free Mg$^{2+}$ ions is approximately 1 mM) and is present in all DNA and RNA activation processes [1]. It was recognized that millimolar Mg$^{2+}$ concentrations are able to stabilize RNA tertiary structures that are otherwise only marginally stable in the presence of high monovalent cation concentration [3].

One of the NA structural arrangements which are specifically stabilized by magnesium ions is a triple-stranded helix. The most common synthetic triple helices are formed from a homopurine–homopyrimidine tract of a normal Watson–Crick duplex which accepts, at proper concentration and environmental conditions, a third pyrimidine strand into its major groove through a Hoogsteen base-pairing scheme [7]. In 1957, Felsenfeld et al. were the first in describing the formation of a complex between two strands of polyU and one strand of polyA in the presence of divalent cations [4].
A few years ago Kankia published a complex study of magnesium effect on polyU-polyA duplexes [6]. It was demonstrated by using UV spectroscopy, isothermal titration calorimetry, ultrasound velocimetry and densimetry that increased magnesium concentration in a mixed solution of polyU and polyA in 1:1 stoichiometric ratio can lead to decrease of duplexes concentration and at the same time appearance of triplexes.

Despite the large set of employed methods, none of them reflects directly details of the structural arrangement like Raman spectroscopy. Our work represents results of Raman measurements on the mixed polyU-polyA 1:1 solvent with gradually increased magnesium concentration and analysis of the spectral set.

2. Experimental

2.1. Samples

The K$^+$ salts of polyadenylic acid (polyA) and polyuridylic acid (polyU) were obtained from Sigma and used without further purification.

A stock solution containing equimolar concentrations of polyU and polyA was prepared in 80 mM cacodylate buffer (pH 6.4) to reach 20 mM final concentration of bases. This mixture was heated up to 90°C and slowly cooled down (during 12 h) to room temperature. In a similar way, reference solutions of 2:1 mixture, pure polyU and pure polyA were also prepared (the last two without temperature annealing).

Six titration solutions containing MgCl$_2$ in concentrations of 0, 10, 20, 30, 40 and 50 mM were prepared in 80 mM cacodylate buffer. NaCl was added to the solutions in the concentrations to adjust the ionic strength of all solutions at the same value of 200 mM.

Samples for the Raman titration experiment were prepared by mixing six aliquots of 20 µl of the corresponding polynucleotide solution with 20 µl of each one of the six MgCl$_2$ titration solutions. So, the final concentration of magnesium and of each polynucleotide (and the ionic strength of the solution) was reduced to the half.

2.2. Raman spectroscopy

Raman spectra were excited by the 532.15 nm line from a Nd-YAG Verdi V2 laser (Coherent) with the power at the sample of 450 mW. Spectra were measured in a 10 µl microcell using a 90° scattering geometry by a Spex 270M spectrograph (Jobin-Yvon) with a 1800 grooves/mm grating and a liquid nitrogen cooled CCD 1340 × 100 detector (Princeton Instruments). Total acquisition times were 25 min, temperature was stabilized at 18°C. Raman shifts of the sample spectra were calibrated by using Raman spectra of a neon lamp recorded after each measurement of the sample.

2.3. Data treatment

Background correction was performed by subtracting water and cacodylate Raman spectra and by subtracting a fifth degree polynomial. Corrected Raman spectra were then subjected to factor analysis using a singular value decomposition (SVD) algorithm that decomposes a set of $N$ spectra, $Y_i(\tilde{\nu}), i = 1, 2, \ldots, N$, into a set of independent (but artificial) orthonormal spectral components $U_j(\tilde{\nu})$: $Y_i(\tilde{\nu}) = \sum_{j=1}^{M} V_{ij} W_j U_j(\tilde{\nu})$. The coefficients $V_{ij}$ quotes relative portion of the $j$th spectral component $U_j(\tilde{\nu})$ in the $i$th original spectrum $Y_i(\tilde{\nu})$ and the singular number $W_j$ stands for the statistical weight of the $j$th
spectral component. The factor space dimension $M$ ($M < N$) represents the minimum number of components $U_j(\nu)$ that are enough to approximate the original spectral set.

3. Results and discussion

The SVD analysis of magnesium titration data has shown that the factor dimension of the spectral set is only two. This means that within an experimental error the spectral effect of the gradually increased magnesium concentration can be represented as a linear combination of only two spectral components shown in Fig. 1(a). The first component represents something like an average spectrum, while the second component reveals the magnesium induced changes on the Raman spectra. Dependence of the spectral change on magnesium concentration can be displayed by the ratio of respective coefficients, i.e., $V_2/V_1$ (Fig. 1(b)).

Observed spectral changes were more intensive and different from those obtained when magnesium was added only to a single-strand polyU or polyA. Because it is known that Raman spectrum of polyU-polyA-polyU triplex differs sufficiently from any combination of polyU-polyA duplex, sole polyU and sole polyA Raman spectra [5], we can easily prove if the observed spectral changes agree with the expected magnesium induced reaction $\text{polyA-polyU + polyA-polyU} \rightarrow (\text{polyA-polyU})\cdot\text{polyU} + \text{polyA}$. Figure 2 shows that the shape of the second SVD component corresponds very well to the difference spectrum constructed mathematically from normalized Raman spectra obtained from solutions without magnesium as

$$(\text{polyA-polyU})\cdot\text{polyU} + \text{polyA} - 2 \times \text{polyA-polyU}.$$ 

This clearly proves that the observed spectral changes reflect the magnesium induced triplex formation from polyU-polyA duplexes. Direct effects of magnesium binding to single-strand polynucleotides are not resolvable in our set of Raman spectra – very probably they are much weaker than those caused by the change in content of duplex, triplex and single-strand structures.

Fig. 1. Results of the SVD analysis. Magnesium titration on polyU and polyA mixed solution with concentration ratio 1:1, in 80 mM cacodylate buffer pH 6.4, the final concentration of bases was 20 mM. (a) First two spectral components, the first component $U_1$ is an average spectrum present in all measured spectra, while the second component $U_2$ represents the magnesium induced changes on the measured Raman spectra. (b) Ratio of the normalization coefficients $V_2/V_1$ showing the dependence of the spectral change on the magnesium concentration.
Fig. 2. The magnesium induction of triplex formation from polyU-polyA duplexes. On the left side, (a) second SVD component obtained from the data analysis of the measured spectra with magnesium; (b) difference spectrum constructed mathematically from normalized Raman spectra obtained from solutions without magnesium. The graphic on the right shows the estimated amount of duplexes and triplexes in every 1:1 polyU-polyA solution with different magnesium concentrations.

The fact that the difference spectrum was constructed from normalized Raman spectra obtained from solutions with the same concentrations of bases allowed us to estimate the amounts of duplexes and triplexes in every 1:1 mixed polyU-polyA solution. The result is shown in Fig. 2 (right graph). It can be seen that magnesium does not cause any noticeable triplex formation until it exceeds concentration of duplex basepairs.

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References

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