Raman spectroscopic studies on the conformational changes of calf thymus DNA induced by Cd^{2+} ions

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We used Raman spectroscopy to study the conformational changes of DNA induced by Cd^{2+} ions in different Cd^{2+} concentrations solution. The experimental results show that when the Cd^{2+}/PO_2^- ratio R increased from 0 to 3.0, the band 835.0 cm^{-1} shifted about 8 cm^{-1}, and the overlapping spectra of 1446.0 and 1461.0 cm^{-1} separated and moved to 1441.0 and 1458.0 cm^{-1}, respectively. This indicates that the conformation of DNA has changed from a "normal" B-form to a "modified" B′-form. At the same time, changes of other bands demonstrate that parts of base stacking collapse and some hydrogen bonds between AT are disrupted, AT base pairs are damaged more larger than GC base pairs.

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Recently, the conformational changes[1−4], condensation properties[5,6] and dynamics properties[7−9] of DNA have become hotspots and the interactions of metal ions with DNA[4−6,10−13] are most interesting. Researchers have found that some lung cancers and prostate cancers resulted from the interaction of Cd^{2+} with DNA. So, to understand this interaction is very important for us to identify the toxic reason and to guard against diseases. Raman spectroscopy can scale the vibrations of molecules and each Raman band corresponds to special vibration energy of molecules. So using Raman spectroscopy, we can identify the structure of DNA and estimate the properties and changes of its chemical bond lengths.

Calf thymus DNA used in this experiment was purchased from Sigma Co., and the used $CdCl_2 \cdot 2.5H_2O$ and NaCl were both in analytical grade. NaCl was dissolved in three-distilled water to 0.2 mol/l. The DNA/$CdCl_2$ mixtures were prepared as follows. A solution of DNA (0.087 mg/μl) was first prepared by dissolving in NaCl solution. Then the appropriate $CdCl_2$ was added to DNA solution with the desired Cd^{2+}/PO_2^- ratios (R=0, 0.5, 1.0, 1.5 and 3.0). All samples were kept at 4 °C for 24 hours and then centrifuged to ensure the formation of a homogeneous solution.

The Raman spectra of the samples contained in capillary tubes were excited by the 514.5-nm line of an Innova 750 argon ion laser (Coherent Co.) (about 200 mW of laser power at the sample) and recorded on a T64000 system (Jobin-Yvon Co., France). The effective spectral resolution was 4 cm^{-1}. Raman spectra were collected at 1.0 cm^{-1} increment with an integration time of 2 s. The reported Raman frequencies are believed to be accurate to within ±1 cm^{-1}. According to Ref. [14], we select Raman band 1578.0 cm^{-1} as an internal standard in the experiment.

Figures 1 and 2 exhibit the Raman spectra of pure DNA and Cd^{2+}-DNA with different Cd^{2+}/PO_2^- ratios, respectively. Figure 3 was extracted from Fig. 2, which displayed the changes of band 835.0 cm^{-1} obviously. Comparing Fig. 1 with Fig. 2, we found when R increased from 0 to 3.0, two prominent changes occurred: 1) Band 835.0 cm^{-1} shifted to 827.0 cm^{-1}. 2) The overlapping bands of 1446.0 and 1461.0 cm^{-1} separated gradually and shifted to 1441.0 and 1458.0 cm^{-1}, respectively. Besides, the intensities or frequencies of other bands also changed.

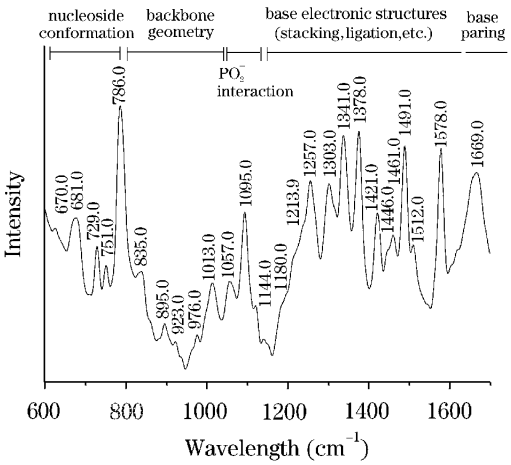

Fig. 1. Raman spectra of pure B-DNA with 0.2-mol/l NaCl solution, the DNA concentration is 0.087 mg/μl.

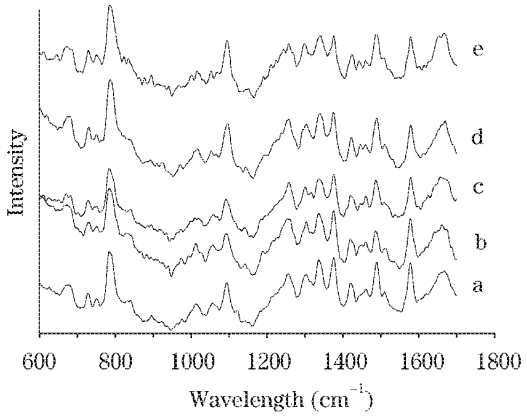

Fig. 2. Raman spectra of pure DNA and DNA complexes with Cd^{2+} in solutions with different Cd^{2+}/PO_2^- ratios: (a) R=0; (b) R=0.5; (c) R=1.0; (d) R=1.5; (e) R=3.0.

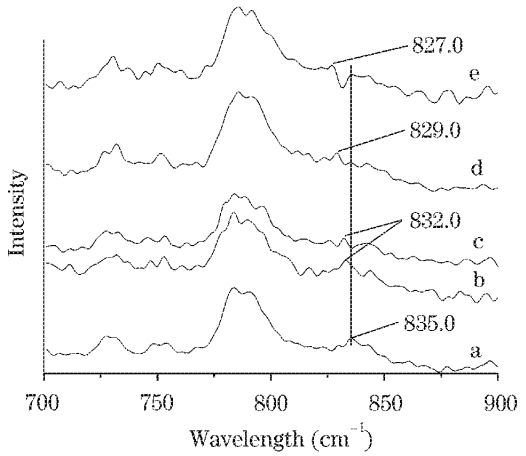

Fig. 3. Raman spectra of pure DNA and DNA complexes with Cd^{2+} in solutions with different Cd^{2+}/PO_2^- ratios: (a) R=0; (b) R=0.5; (c) R=1.0; (d) R=1.5; (e) R=3.0. Extracted from Fig. 2.

The phosphate groups of B-DNA are known to give rise to three prominent Raman bands (786.0, 835.0 and 1095.0 cm^{-1}) that serve as reliable indicators of B-form structure[10,15]. The strong band 786.0 cm^{-1}, which is due to 5'C-O-P-O-C3' network, is always overlapped by the symmetric vibration stretching of the OPO[15–17] and the breathing mode of cytosine ring (780.0 cm^{-1})[9,10,17]. From Fig. 2, we find that when R is equal to zero, $I_{786.0}/I_{1578.0}$ = 1.02, when R increases to 0.5, 1.0, 1.5 and 3.0, $I_{786.0}/I_{1578.0}$ =1.10, 1.00, 1.19 and 1.14, respectively. These changes show that with the increase of R, the intensity of 786.0 cm^{-1} has increased but this enhancement was irregular with R. So we conclude that with the increase of R, the compositions of DNA have changed and the percentage of GC increased[9,10,17].

Band 835.0 cm^{-1}, which is also owing to 5'C-O-P-O-C3' network[10], is sensitive to subtle conformational variants within the B family structure. Figure 3 shows that when R increases from 0 to 3.0, the relative marker 835.0 cm^{-1} shifts to 832.0, then to 829.0, and at last to 827.0 cm^{-1}. According to Refs. [10,15,16], we know that Cd^{2+} ions have interacted with DNA and made the total percentage of GC increase. At the same time, the groove dimension of AT and GC became more narrow and minor[18]. All these resulted in the conformational changes of DNA. But the backbone geometry of DNA was still subjected to B family, according to Refs. [9,17], we know such DNA is a "modified" B′-type backbone.

The strong band 1095.0 cm^{-1} is due to the symmetric stretching vibration of the PO_2^- moiety[5,10,14,19]. From Fig. 2, we can see that its frequency was almost invariable, while with the increase of R, the structure of B-DNA has not changed into A-DNA[10,15–17], but its intensity and width enhanced. Purely electrostatic interactions of PO_2^- with Cd^{2+} are not believed to appreciably modify, either in intensity or in frequency. More likely, the observed intensity increasing and the Raman width broadening were caused by much more direct type of interaction with one particular oxygen atom of PO_2^-, giving a –P(=O)-O-Cd^{2+} type of complex. The formation of a $-PO_2^- \cdots Cd^{2+} \cdots$ N7 (purine) chelate was previously suggested[13].

There are also some weak-to-moderate intensity bands which are sensitive to the geometry and secondary structure of DNA[12]. The most noticeable bands were 1446.0 ($CH_2\delta$) and 1461.0 cm^{-1} (5'-$CH_2\delta$)[10,16]. In Fig. 1, these two bands overlap with each other and are difficult to distinguish. But in Fig. 2 they are easy to distinguish, which has not appeared in previous experiments. In Fig. 2, when R is under 1.5, this separation is ambiguous; but when R increases to 1.5, it becomes obvious and the two bands move to 1444.0 and 1458.0 cm^{-1}, respectively; when R increases to 3.0, the forth moves to 1441.0 cm^{-1} but the latter retains invariable. Their intensities increase continuously. These changes suggest that the H-bonding at the C=O sites weakened[10,20]. Bands such as 895.0 (d), 923.0 (d), 976.0 (d) and 1013.0 cm^{-1} (T, G, C) all generate red shifts (2, 7, 7 and 3 cm^{-1}, respectively) and show changes in intensity. These changes also indicate that the geometry and secondary structure of DNA have changed[10].

Cd^{2+} ions can interact not only with PO_2^- but also with DNA bases. It is well-known that the vibration of DNA bases is mainly collected in the range of more than 1200 cm^{-1}, and in this range the changes of bands 1303.0 (A), 1341.0 (A, G) and 1378.0 cm^{-1} (A, T, G)[10] were most attractive. In Fig. 2, when R increases from 0 to 3.0, these high and acute bands all decrease prominently in intensity and change into low and broad bands. The loss in intensity of these three bands implies that the percentage of GC increased[21]. According to the Raman hypochromism effect[9–11,19], we think that parts of base stacking collapsed. On the contrary, the intensity at 1491.0 cm^{-1} (G, A) increased largely, which demonstrates that Cd^{2+} had bound to G at N7 site[12,13] and this binding strengthened the energy of all hydrogen bonds between GC base pairs and reinforced the stability of GC base pairs[13,22].

The alteration in intensity of 1669.0 cm^{-1} also proved that the interaction of $Cd^{2+}$with DNA has caused the increasing of the percentage of GC. The increasing in intensity of band 1669.0 cm^{-1} (T, G, C)[9,11,15] with R shows that Cd^{2+} has interacted with C=0,NH NH_2 of A[12] and this interaction led to the loss of amidogens[9,12] and the instability of B-DNA structure. Furthermore, this also made parts of hydrogen bonds between AT base pairs disrupted[9,12]. The results in intensity of 670.0 (T), 681.0 (G) and 729.0 cm^{-1} (A)[10] testified this too. In Fig. 2, these bands all change in intensity. According to Refs. [12,21], we know that parts of base stacking collapsed and the percentage of GC enhanced but the deoxyribose (C2'-endo/anti) conformation still kept C2'-endo/anti fold mode[9].

In conclusion, Cd^{2+} ions have interacted with DNA and the degrees of these interactions are different with different Cd^{2+} concentrations in DNA-solution. Cd^{2+} ions can interact not only with PO_2^- but also with DNA bases, which are clear in our experiment. The bindings of Cd^{2+} to PO_2^- mainly depend on the chelating with PO_2^-, and form $-PO_2^- \cdots Cd^{2+} \cdots$ N7 (purine) chelates. Its interactions with G at the N7 site can largely reinforce all hydrogen bonds between GC base pairs and the energy of these bonds, and thus facilitate the stability of GC base pairs. Cd^{2+} ions can also cause parts of base stacking of AT bases to collapse, disrupt a part of hydrogen

bonds between AT base pairs and make AT base pairs unpaired, and thus lead to the loss of amidogen moiety and the damage of AT base pairs. During the interaction of Cd^{2+} ions with DNA, the backbone geometry and secondary structure of DNA change, B-DNA is no longer a "normal" B-DNA but a "modified" B′-DNA. AT base pairs are damaged larger than GC and the percentage of GC increases.

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