Chinese Chemical Letters Vol. 16, No. 1, pp 71-74, 2005 71
http://www.imm.ac.cn/journal/ccl.html

Preliminary Study on Cordycepin-DNA Interaction by Raman Spectroscopy

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Abstract: The interaction of cordycepin with calf thymus DNA was investigated at physiological pH with drug/DNA molar ratio of 8. The Raman spectroscopy results indicated that the intercalation of high concentration cordycepin and the interaction of cordycepin with PO_2 group led to a major reduction of B-form DNA structure in favor of A-form DNA.

Keywords: Cordycepin, calf thymus DNA, Raman spectroscopy, conformation.

Cordycepin, a nucleoside analogue 3′-deoxyadenosine, has a broad spectrum of biological activity[1,2]. The present studies focus on the interaction of cordycepin and mRNA in cellular level[3,4]. Anticarcinogen can be related to the complexation with DNA. To understand the structure of cordycepin-DNA complex has major biological importance, and thus, this investigation was undertaken. To our knowledge, there is no report on using Raman spectroscopic technique in this aspect. In this paper, the effects of cordycepin-DNA on secondary structure of biopolymer and the helix stability are evaluated.

Calf thymus DNA and cordycepin were purchased from Sigma chemical (St.Louis, Mo, USA). DNA was originally dissolved in 0.01 mol/L (pH 7.0) KNO_3 solution at 4 ℃ for 24 h with occasional stirring to ensure formation of a homogeneous solution. Its concentration was determined by the Shimdzu UV-240 Spectrophotometer (Shimadzu Corp. Kyoto, Japan) at 260 nm (ε_{260}=6600L · mol^{-1} · cm^{-1}). A stocking solution of cordycepin was 1×10^{-2} mol/L solved in 0.01 mol/L KNO_3 solution.

Calf thymus DNA and cordycepin solution were mixed to the desired drug/DNA molar ratio (rb=[cordycepin]/[DNA]), and diluted to the needed final DNA concentration with the above KNO_3 solution. After initial mixing of cordycepin and DNA solutions for 4 h, the sample was sealed in a glass capillary and mounted in the sample illumination of the RM 2000 microscopic confocal Raman spectrophotometer(Renishaw Co. England). The experimental conditions were as follows: exiting line 514.5 nm, power 50% (100%=4.07 mw); a microprobe×20 objective, diameter of photospheric 5 μm (20 obj); focus on the nucleus; scanning ranges 100-1800 cm^{-1} , room temperature (18±2°C).

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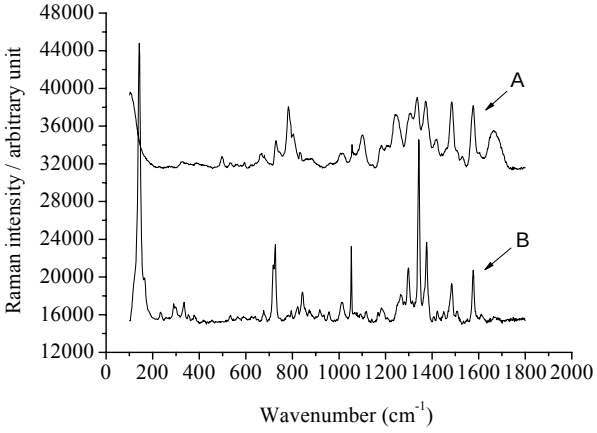
Table 1 Raman spectra of DNA and assignments

DNA frequency/cm	Assignment
498	deoxyribose—phosphate
534	A
595	G, C
668	T, G
680	G
729	A
787	O-P-O symmetric stretch
805	A-type diester stretch
834	B-type diester stretch
1007	C-O stretch
1055	C-O stretch
1095	O-P-O symmetric stretch
1145	deoxyribose-phosphate
1237	G
1306	A
1336	A
1373	T, A,G
1420	A, G
1484	G, A
1530	C,G
1577	G,A
1644	C

Figure 1 Raman spectra of free calf thymus DNA and the cordycepin-DNA complex



(A) Spectrum of free calf thymus DNA. (B) Observed difference spectrum of cordycepin-DNA complex, rb=8. Spectra shown were accumulated averages of 3 exposures of 100 s each in KNO_3 solution in the region of 1800 to 100 cm^{-1}.

The Raman spectra of free calf thymus DNA was shown in **Figure 1-A**. The detailed assignments of the major bands listed in **Table 1**. A, G, C and T listed in **Table 1** indicated the vibration characteristics of adenine, guanine, cytosine and thymine bases, respectively[5-7]. The key structural markers of DNA are: 681 cm^{-1}, diagnostic of C2′ -*endo* sugar pucker and *anti* glycosyl torsion[8]; 732 cm^{-1}, diagnostic of C2′ -*endo/ anti*

deoxyadenosine[9]; 790 cm^{-1} and 831 cm^{-1}, diagnostic of phosphodiester torsions α/δ in the $gauche^-/gauche^-$ range[10]; 1095cm^{-1}, phosphodiester O-P-O stretching[9]; 1420cm^{-1}, indicative of C2´ H_2 moieties in the B-DNA conformation[5]. All above five points proved that the backbone structure of the double-stranded calf thymus DNA remained orderly in B-form.

The shoulder line appeared at 805 cm^{-1}, which related to the stretching vibration of O-P-O phosphodiested bond, is sensitive to spatial structure of single-stranded DNA, and is the evidence of A-form DNA. As influenced by sugar pucker and glycosyltorsion, the band at 668 cm^{-1} belonging to the breathing vibration of guanine ring indicated the transformation from glycosidic bond to fluranose ring in guanosine, and it was also diagnostic characteristics of canonical A-form DNA.

According to the experimental data, it can be concluded that calf thymus DNA exists mostly in B-form, the KNO_3 solution of calf thymus DNA meanwhile in local fragment is in A- form probably.

Raman spectra of calf thymus DNA bond to cordycepin obtained by subtracting cordycepin from the spectrum of cordycepin-DNA complex. The bands belonging to various groups of bases had respective changes. The band intensities associated with the guanine and cytosine ring at 1237 cm^{-1}, 1251 cm^{-1}, 1422 cm^{-1}, 1483 cm^{-1}, 1530 cm^{-1}, 1576 cm^{-1} decreased in variant degree, while Raman bands to the adenine ring at 1301 cm^{-1}, 1335 cm^{-1} and the band to the vibration of thymine, adenine and guanine ring at 1372cm^{-1} represented hyperchromic effect and slight shift[9]. These changes were likely due to electrostatic interactions of cordycepin with the active site, which may include π-π stacking between adenine ring of cordycepin and corresponding DNA bases. The reduction in the intensity and shifting of the Raman bands, at 498 cm^{-1}, 834 cm^{-1} and 1146 cm^{-1}, were assigned to the special characters of backbone phosphate(**Figure 1-B**). Great change was also observed to the band at 1095 cm^{-1} belonging to the symmetrical vibration of PO_2 group. Those estimated that the regular double helix structure of DNA was destructed badly, and also meant that drug-PO_2 interaction may occur in these cordycepin-DNA complexes. The apparent descent of the C=O stretching vibration of conjugate base at 1667 cm^{-1} proved the same conclusion. It was related to the breakage of inter-base hydrogen bonds and implied the double helix structure was broken into single-stranded DNA. The Raman bands in the 781-1100 cm^{-1} internal were attributed to the vibration of deoxyribose or co-effect of deoxyribose and PO_2 group, for example, the band at 844 cm^{-1} assigned to the stretching vibration of 5-phosphate-deoxyribose. The same hyperchromic effect existed in the bands of the guanine and adenine ring at 1592 cm^{-1}, the cytosine ring at 1614 cm^{-1}, the C=O group in cytosine at 1650 cm^{-1}, the C=O group in thymine at 1670 cm^{-1}, the C=O group in guanine at 1710 cm^{-1}, meanwhile, the increment of the Raman bands at 1008 cm^{-1} and 1053 cm^{-1} illustrated the stretching vibration of C-O group in deoxyribose was also increased.

In summary, high rb value of cordycepin-DNA binding resulted in decrease of B-form DNA structure and favor of A-form DNA. It was suggested that stacking force and the hydrogen bond between base pairs were destructed and a part of B-form DNA became single-stranded. The backbone phosphate, deoxyribose and base pair were changed correspondingly.

Acknowledgments

Project 3037014 was supported by the National Natural Science Foundation of China.

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Received 3 December, 2003