

Superhelical Spatial Conformation on Circular DNA in Aqueous Solution

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Abstract : The circular DNA has been shown a regular superhelical spatial conformation. The Raman spectra of bacterial plasmid DNA in aqueous solution were recorded. Both diagnostic bands at the 854 and 1083 cm^{-1} reflecting the vibration state of the tertiary structure of the DNA main backbone are usually hard to find in the Raman spectra of the linear DNA molecule and their assignments have been discussed. The intensity of the peak at 969 cm^{-1} , corresponding to the C'—C' stretching vibration of the deoxyribose, is between that of calf thymus DNA in aqueous solution and in fiber ($0.46 < 0.83 < 1.10$). As a result of analyzing base stacking, the existence of the superhelical spatial conformation represents both the increases in the staking reaction activity of thymine residues in the DNA molecule and the damage of a considerable amount of the Watson-Crick type hydrogen-bonding in the AT base pairs. Meanwhile, the characteristic band at 1378 cm^{-1} , corresponding to C—N and C=O group vibration, indicating the composition of H-bond of the thymine, has markedly Raman hypochromism. It is a sufficient basis of confirming the tertiary structure of circular DNA molecule with Raman characteristic bands.

Key words : plasmid DNA ; tertiary structure ; Raman spectroscopy ; superhelix

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0 Introduction

Apart from the chromosome DNA in the prokaryotic cell, plasmid DNA is a covalent closed circular DNA (i. e. cccDNA) molecule. Its conformation differs from that of a linear molecule. The closed circular molecule has not only the secondary structure of a right hand helix, but also tertiary structure for the special superhelical conformation. Whether it is linear, opened circular, or closed circular, all DNA molecules in the physiological environment are in a superhelical state. However, their conformations are different. The superhelical conformation of a closed circular molecule is special. The closed circular

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molecule could form a bracelet state or negatively twisted superhelix. During the DNA purification ,the linear or opened circular DNA molecule hardly keeps its natural superhelical state. The cccDNA molecule however could maintain as the same structure as that inside the cell ,even after it was handled through getting out of protein and precipitating in alcohol again and again. The experiments of topoisomerase and intercalating fluorescent molecule are all confirmed^[1].

In the study of DNA spatial conformation with Raman spectroscopy ,only the information of the conformation of nucleotide unit and the secondary structure of phosphate deoxyribose main chain can be obtained from the literatures. Bacterial chromosome DNA also is a double stranded circular molecule as the same as plasmid DNA. However ,its molecular weight is bigger than plasmid DNA ,thus ,it could very easily be broken into a linear molecule during purification ,which can be confirmed from its Raman spectra because its Raman spectra after purification are as the same as that of the linear DNA molecule^[2]. It was reported that the Raman spectra in the region from 600 to 900 cm^{-1} are quite sensitive to the second spatial conformation of DNA. However ,the conclusion was obtained only from the comparison between double stranded linear DNA and double stranded opened circular DNA not including the data for the closed circular molecule^[3]. In addition ,their results do not show the obvious difference between the two kinds of molecules in this region. It is well-known that the opened circular DNA molecules have partially lost the capacity to form superhelical structure with the internal stress to be released. The double stranded linear molecules are also lack this stability structure. When the plasmid DNA of a double-strand closed circuit is forming the tertiary structure ,the internal stress of molecules was changed ,so the vibration energy of relevant covalent bonding is different from that of the linear or opened circular molecule. These changes can be measured by Raman spectroscopy. However ,until now ,there are no any literature regarding Raman spectra of the pure plasmid DNA according to our knowledge.

The plasmid is quite important substance in gene engineering. If the properties in its spatial conformation are well understood ,it will be favorable to elucidate many active mechanisms. The superhelical plasmid tertiary structure from the Raman data of pBR322 recombinant plasmid DNA has been reported^[4]. Some results have been obtained from previous studies^[4~7]. Firstly ,there are two intensive peaks at 854 cm^{-1} and 1083 cm^{-1} except the characteristic bands of B-form at 830 cm^{-1} and 1096 cm^{-1} due to the secondary structure of the main chain^[4]. All of the above peaks are related to the phosphodiester band stretching vibrations. Secondly ,the band at 1378 cm^{-1} indicating vibration of the base ring in-plane is obviously lower than that of the linear DNA molecule^[5~7]. Finally ,the intensive Raman peaks located in the region from 1600 to 1700 cm^{-1} were assigned to the double bond of the ketone group. The above characteristics of the Raman spectra of the plasmid DNA were different from that of the linear DNA. Thus ,it can be suggested that the tertiary structure state of natural DNA can be measured Raman spectra with high sensitivity.

1 Materials and methods

1.1 Materials

The pBR 322 recombinant plasmid DNA was purchased from the Hua Mei Biology Engineering Company, China. It was transformed into the E. Coli JM109 strain cell and then amplified in our

laboratory. CsCl was from Shanghai Reagent No.1 Factory and ethidium bromide was purchased from the Fluka Company.

1.2 Extraction and purification of plasmid

Bacteria culture ,extraction and purification of plasmid were carried out according to routine method^[8]. The product was a plasmid DNA with the identical molecular weight and circular conformation which was proved by 0.8% agarose gel electrophoresis analysis. The product concentration was 0.4μg/μL with the quantitative measurement of the λ bacteriophage DNA concentration gradient standard calibration curve.

1.3 Raman spectra

Raman spectra were measured with an HRD-2 double grating monochromator(Jobin Yvon Co. , France) with an adapted RCA-31034 photomultiplier and an INNOVA 70 argon ion laser(Coherent Co. , USA). The excitation wavelength was 514.5 nm with 150 mW power. The monochromator slit width was 500μm and the scanning speed was 1 cm⁻¹/s. The spectral resolution was ± 2 cm⁻¹. The signals were collected with a 90 ° geometry. The 10 μL aqueous solution of plasmid DNA was infused into a glass capillary(1 mm i. d.) with a piece of Parafilm to seal it. The experiment was performed at 20℃ .

2 Results and discussion

2.1 The spatial conformation of the secondary structure of the phosphate-deoxyribose backbone

Compared with the Raman spectra of the double stranded linear DNA , the characteristic bands of the secondary structure of the deoxyribose-phosphate backbone are not obviously changed in the Raman spectra of plasmid cccDNA. From the presence of the Raman bands at 685 ,799 ,830 ,1 096 cm⁻¹ in Figure 1 , it can be confirmed that the plasmid cccDNA has B-form structure in solution^[5,7,9].

The main peaks in the Raman spectra of plasmid cccDNA in the aqueous solution were tentatively assigned according to the literature^[10,11]. The results were listed in Table 1. The relative intensities of the Raman peaks of deoxyribose in

Table 1 Raman spectra of the plasmid cccDNA in aqueous solution

Raman shift/ (cm ⁻¹)	Tentative assignments	Raman shift/ (cm ⁻¹)	Tentative assignments
514	dG	1 145	deoxyribose
671	dG	1 184	dT ,dC
685	dG	1 240	dT ,dC
724	dA	1 258	dC ,dA
799	dT ,dC ,B-type main chain	1 301	dA ,dC
	OPO symmetric stretching	1 316	dG
830	B-type main chain	1 341	dA
	OPO asymmetric stretching	1 368	dA
854	main chain backbone	1 428	deoxyribose
891	deoxyribose	1 443	deoxyribose
920	deoxyribose	1 453	deoxyribose
939	deoxyribose	1 463	deoxyribose
969	deoxyribose	1 481	dA ,dG
978	deoxyribose	1 518	dA
1 022	deoxyribose	1 590	dA
1 052	deoxyribose	1 627	ketone group
1 083	main chain backbone	1 662	dT ketone group
1 096	B-type main chain , PO ₂ ⁻ stretching	1 688	dG ketone group

Note : The main component in each peak has been assigned on the table.

various DNA were shown in Table 2 ,respectively.

2.2 The vibration model of the deoxyribose CO ,CC and CH

The Raman spectra in the region from 880 to 1 065 cm⁻¹ correspond to the stretching vibration pattern of deoxyribose C ' - O - C ' and C ' - C ' .It is well-know that the C - H vibration exists for purine C8 ,dA C2 ,Pyrimidine C6 and dC C5 in the base part of the nucleotide and also for the C '1 ,C '2 ,C '3 , C '4 of deoxyribose .In addition ,the C '5 has the vibration of C 'H₂ . It was reported that the peaks at 1 428 ,1 443 ,1 453 ,1 463 cm⁻¹ are attributed to C - H and CH₂ of both the base part of the nucleotide and deoxyribose^[12 ~ 15] .From the data in Table 2 ,it was found that the above four vibration models of the CH , CH₂ in the cccDNA were stronger than that of linear DNA .Thus ,it may be inferred that these peak mainly belong to the C 'H ,C 'H₂ vibration of deoxyribose ,particularly for the peak at 1 463 cm⁻¹ .

Table 2 Relative peak intensity of the deoxyribose vibration in different DNA molecules

Raman shift/ (cm ⁻¹)	Tentative assignments	cccDNA in aqueous solution	ctDNA in aqueous solution	ctDNA in fibre	P22 DNA	Dodecamer in aqueous solution	Dodecamer in fibre
895 ± 4	C - O stretching C - H deformation	0.69	1.04	0.77	0.24	0.15	0.23
924 ± 4	C - O stretching	0.67	0.72	0.77	0.18	0.12	0.19
944 ± 5	C - O stretching	0.79	0.42	0.83			
963 ± 6	C - O ,C - C stretching	0.83	0.46	1.10			0.17
975 ± 3	C - O ,C - C stretching	0.60	0.79		0.09		
1012 ± 5	C - O stretching		0.68	1.27	0.43	0.44	0.45
1023 ± 1	C - O ,C - C stretching	0.66		1.03			
1056 ± 4	C - O ,C - C stretching	0.62	0.68	1.07	0.40	0.31	0.53
1424 ± 4	CH ,CH ₂ deformation	0.64	0.58	0.48	0.62	0.46	0.62
1445 ± 3	CH ,CH ₂ deformation	0.76	0.54	0.53	0.25		0.47
1452 ± 1	CH ,CH ₂ deformation	0.86	0.65	0.58			
1464 ± 4	CH ,CH ₂ deformation	0.71	0.63	1.07	0.42	0.29	0.64

Notice :The intensity of each peak band is normalized against the PO₂⁻ stretching vibration peak and value assigned to 1.00 in the sample.The Raman data of the ctDNA ,the P22 DNA and the dodecamer are from the literature^[5 ,6 ,11 ,12 ,13]

The peak at 969 cm⁻¹ does not exist in RNA or oligoribonucleotide ,thus ,it is concluded that this peak corresponds to the C ' - C ' stretching vibration with the C '2 deoxygenation and it contains the in-plane rocking deformation of C 'H₂ of the C '5 in deoxyribose^[14] .The relative intensity of this peak for cccDNA is between that for calf thymus DNA in the aqueous solution and in the fiber (0.46 < 0.83 <

1.10). Because linear DNA in the aqueous solution has almost no regular tertiary structure and DNA in fibers is in the concentrated state with an undefined tertiary structure. Thus, the relative intensity of the peak at 969 cm^{-1} may reflect the superhelical state of DNA in aqueous solution. Of course, more work should be done for this conclusion.

In the Raman spectra of the seven natural DNA, such as calf thymus DNA, three kinds of the bacteria DNA (i. e. *Escherichia coli*, *Clostridium perfringens*, *Bacillus subtilis*), three kinds of the bacteriophage DNA (i. e. P22, PRD1, ϕ X174) and fourteen artificial synthesis polynucleotides, i. e. d(CGCAAATTGCG), d(CCCCGGG), d(CGCGCG), d(CGCATGCG), d(GGGATCCC), d(T_4G_4), d(CGCGTG), poly(rA)poly(dT), λ m⁵CGTAm⁵CG, poly(dA·dT)poly(dA·dT), poly(dG·dC)poly(dG·dC), λ GCG λ CGC, λ phage O_L1(19 basepair) λ phage O_R3(17 basepair), none of them has as the same vibrations of deoxyribose as that for plasmid cccDNA. Thus, it can be deduced that the deoxyribose conformation has an important change when the cccDNA formed the superhelical structure.

It was reported that the band at 891 cm^{-1} indicates the C'-H deformation vibration of the deoxyribose β isomer and the band at 917 cm^{-1} belongs the asymmetrical ring vibration of the β isomer^[16]. The above two peaks were also observed in Figure 1. Thus, it was confirmed that the β isomer conformation of deoxyribose in cccDNA in the aqueous solution also exists. It may indicate that the β isomer conformation of deoxyribose in the tertiary structure is the same as that in the linear structure.

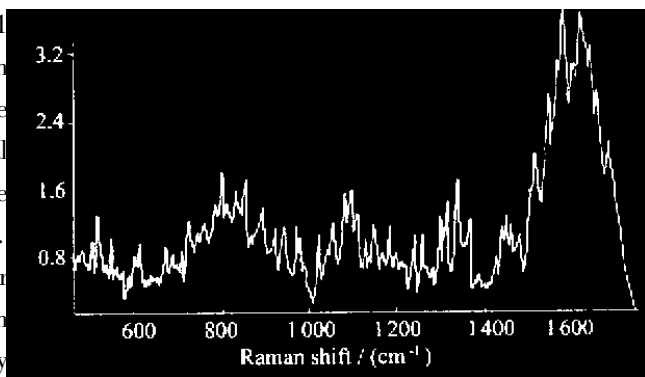


Fig.1 Raman spectrum of plasmid cccDNA in aqueous solution

The C'H₂ vibration mode of the deoxyribose phosphate backbone is sensitive to the secondary structure in the spatial conformation analysis of the linear DNA molecules. The peak for the C'H₂ vibration mode is located at 1422 cm^{-1} in the B-DNA, but it appears at 1425 cm^{-1} in the Z-DNA. The weak peak at 745 cm^{-1} in the Raman spectrum is also related to the Z-DNA^[5]. Thus, the existence of the peaks at 1428 and 745 cm^{-1} in Figure 1 indicates that there is part of the Z type conformation in the cccDNA.

2.3 OPO phosphodiester bonding vibration mode

Because both the Raman bands at 830 and 854 cm^{-1} appeared in the Raman spectrum of cccDNA, it should be make sure if two peaks originate from in proteins as the impurity. It was reported that when there is the tyrosyl Fermi resonance doublet, the peak at 644 cm^{-1} for tyrosine vibration mode should also appear^[4]. However, no peak at 644 cm^{-1} in Figure 1 was observed. On the other hand, there were no strong peaks of the protein amide III and the protein amide I which appeared in the region from 1225 to 1300 cm^{-1} and from 1650 to 1675 cm^{-1} , respectively (Table 3). Therefore, the peak at 854 cm^{-1} did not belong to the tyrosine, in turn, there is no protein impurity or the TE buffer system^[18].

Table 3 Relative intensity of ring in-plane vibration bands on the three bases

Raman shift/ (cm^{-1})	Tentative assignments	Plasmid cccDNA	P22 phage DNA	Calf thymus DNA	ϕ X174 RF III dsDNA
685 ± 4	dG	0.38	0.34	0.41	0.43
727 ± 3	dA	0.45	0.35	0.44	0.43
1240 ± 2	dT	0.50	0.50	0.50	0.50
1301 ± 1	dA	0.54	0.55	0.62	0.60
1342 ± 3	dG ,dA	0.76	0.82	0.88	0.85
1378 ± 4	dT ,dA ,dG	0.18	0.78	1.03	1.00

Note : All kinds of the base characteristic mode were normalized against the peak at 1240 cm^{-1} of the deoxythymidine residues^[12]. The difference between closed ring molecules and linear molecules have been shown. In some linear molecules ,such as the calf thymus DNA and the duplicating III mode ϕ X174 dsDNA were the samples in solution^[9]. But the linear double chain DNA of the mouse typhoid fever salmonella typhimurium P22 virion was the sample after the package nucleocapsid^[6]. P22 virion data has been used in order to compare the superhelical molecules with the high assembling linear molecules. The involved mode of dA ,dG ,dT are listed. The cytosine signal was not shown because it usually was very weak or its change only was due to the acid-base degree^[22].

The phosphodiester mode band at $800 \sim 840\text{ cm}^{-1}$ was considerably affected by three torsion angles in the main chain $\angle(\text{P}-\text{O}5')$, $\angle(\text{O}5'-\text{C}5')$ and $\angle(\text{C}5'-\text{C}4')$ which has a configured ranking of g^-tg^+ . The mode band at $850 \sim 860\text{ cm}^{-1}$ represents the conformation of trans-trans-trans (t-t-t)^[17,19]. The t-t-t conformation can obviously increases the purines-purines base stacking reaction among the DNA single strands^[17,20]. Appearance of the relatively intensive peak at 854 cm^{-1} indicated that there is a high percentage nucleotide located in the t-t-t conformation(Figure 1). The abundance of purine base stacking among the DNA single chains can certify that it was related with the superhelix.

The peak intensity in the region from 830 to 854 cm^{-1} became strong as wave numbers increased , which indicated that the size of the minor grooves became narrow in the DNA double spiral conformation^[6]. However it does need to be certified by other experiments in the plasmid negative superhelical structure research.

The OPO backbone $\angle(\text{P}-\text{O}5')$ and $\angle(\text{O}3'-\text{P})$ were g^-g^- configured conformation in the regular type-B DNA. The greater the limit of the torsion angles ,the wider the peaks of type-B backbone mode and the position of the peaks move to the high wavenumber^[17]. So the peak at 854 cm^{-1} would be the wider torsion angle limit than the regular type-B mode DNA ,which was concerned with the superhelical conformation of plasmid.

2.4 PO₂⁻ group vibration mode

It can be observed from Figure 1 and Table 1 that the relative intensity of the peak at 1096 cm^{-1} is lower than that of the linear molecule. The peak at 1096 cm^{-1} is related to the vibration of the PO₂⁻ group. It is documented that when the DNA molecules gather ,the peak intensity of the PO₂⁻ group descends. It is because the negatively charged DNA is quite concentrated. It caused to increase the cation density in the solution near DNA molecules and then its electrostatic shield decrease the polarization rate of the PO₂⁻ stretching vibration^[6]. Therefore ,the speculation for this was related to the tertiary structure similar to that of the DNA molecules in the concentrated state.

It was usually hard to find the peaks at 854 and 1083 cm^{-1} in the Raman spectrum of DNA except in two kinds of oligonucleotide crystals^[19]. However ,they appeared in the Raman spectrum of plasmid

DNA. It was explained that there was a similarity between the plasmid superhelical structure and the crystallization of DNA in the spatial conformation. The peak at 1083 cm^{-1} has the tentative assignment for the unusual B-type or modified B-type backbone^[20]. There are both bands at 1083 and 1096 cm^{-1} in the Raman spectrum (Figure 1). This indicated that the later is the characteristic mode for the secondary structure. The former might be the secondary structure closely correlated with the tertiary structure. The strong peak at 1080 cm^{-1} also appears in the Raman spectrum in the ultraviolet resonant excitation test, but it took the place of the secondary structure characteristic mode near 1100 cm^{-1} ^[21]. The PO_2^- and other groups could produce the H-bond drawing assistance from water molecules, which caused the part of the original vibrating mode to shift to the low wave number direction. The appearances of both peaks at 1083 and 1096 cm^{-1} indicated that the partial superhelical PO_2^- groups may be formed through the action of the H-bond from the water bridge with other groups, which are not adjoining to PO_2^- groups.

2.5 Base stacking action

The in-plane vibration of the base ring is involved with base stacking action. The base stacking state disappears when the double chain becomes the single chain. It increases the peak intensity. The relative intensities of the in-plane base ring peaks are shown in Table 3. All kinds of the base characteristic modes were normalized against the peak at 1240 cm^{-1} of the deoxythymidine residues^[12]. The data of P22 virion, a high assembling linear molecule were used in Table 3 in order to compare with that of the superhelical molecules. In Table 3, the involved mode of dA, dG, dT are listed. The cytosine signal was not shown because it usually was very weak or its change was only due to the acid-base degree^[22].

From Table 3, the contents of the plasmid DNA is the same as the other three kinds of molecules on the whole terms, except the peak at 1378 cm^{-1} , proving that the action of base stacking in the plasmid superhelical DNA had not changed appreciably. The peak at 1374 cm^{-1} is the marking peak of the DNA reacting activity^[23]. Its peak intensity decreases due to the metal ion action and H-bond state. It is quite sensitive to the dT change^[20] and corresponds to $-\delta\text{N}_3\text{H}(48) - \text{C}_4\text{O}(27)^{10,11}$ vibration mode which was two bonds of sharing H-bond formation. The decrease of its energy results in the peak intensity to descend, indicating that some H-bonds are damaged and changed near the dT side. The change between AT H-bond states was confirmed by the shift of the C_4O peak of dT against high wave number (1663 cm^{-1}) in the region of the diagnostic double bond state^[21]. (Figure 1)

2.6 Vibration mode at the double bond region

The peak intensity at the double bond region from 450 to 1750 cm^{-1} is very high (Figure 1). The wave number of the $\text{C}_2=\text{O}$ vibration of dG shifts to a lower site (1689 cm^{-1}) because of base pairing, which illustrated that the pairing of GC is kept at normal in the plasmid superhelical structure. The appearance of the peak at 1482 cm^{-1} , indicating of an N7 H-bond-state-mode of dG, showed the normal base pairing, but the peak intensity decreases, indicating that the polarization rate becomes weaker. Because there are some changes of the H-bond state, the peak position of the $\text{C}_4=\text{O}$ vibration mode of dT mentioned in section 2.5 shifts to the high wave number. Comparing with the Raman spectra of linear molecules, there is a quite difference in the peak position and the peak intensity of the plasmid spectrum. It revealed the effect from the superhelical state.

As mentioned above, through the analysis for the characteristic vibration modes of phosphate

deoxyribose backbone ,in plane base ring and ketone group with the technique of Raman spectroscopy , some information about the superhelical spatial conformation can be obtained.

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水溶液中的环状 DNA 超螺旋空间构象

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[摘要] 质粒 DNA 水溶液的喇曼光谱研究表明 ,854 和 1083 cm^{-1} 特征峰反映了 DNA 主链骨架三级结构的振动状态 ,表征脱氧核糖中 C—C 伸缩振动的 969 cm^{-1} 峰 ,其相对强度值介于线性 DNA 在水溶液和在纤维状态下所得数值之间 ($0.46 < 0.83 < 1.10$) ;DNA 中胸腺嘧啶的堆积反应活性增加 ,A—T 间氢键能减弱。这些参数是辨认环状 DNA 分子三级结构的有效依据。

[关键词] 质粒 DNA ;三级结构 ;喇曼光谱 ;超螺旋

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