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Line shape analyses for water ^{17}O NMR quintet observed in a bacteriophage Pf1 solution at different temperatures

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Abstract

Partially resolved ^{17}O NMR quintet was observed in a filamentous bacteriophage Pf1 solution at 70 °C with a quadrupole splitting approximately 100 Hz. As the temperature decreased, the resolution was reduced but the line shapes were still indicative of residual quadrupole splitting. Line shape analyses were performed using the quadrupolar relaxation theory for spin 5/2. The contribution to the residual quadrupole splitting from the electric field gradients stemming from the phage filaments, which were oriented in the magnet, was taken into account. As a result, the observed ^{17}O spectra at different temperatures were simulated and the hydration number of the phage DNA was determined.

Keywords: ^{17}O NMR; Quintet; Residual quadrupole splitting; External electric field gradient; Bacteriophage; Hydration number

1. Introduction

The isotope ^{17}O of oxygen, with spin $I=5/2$, is one of the commonly investigated nuclei in NMR [1]. Its applications to biology include the recent investigation of hydration interaction of DNA and proteins [2–8], which can be traced back to early years [9,10]. Like other quadrupole nuclei, in oriented mediums water ^{17}O also shows residual quadrupolar coupling [11–18]. In some liquid crystals water ^{17}O did show well-resolved quintet with a quadrupole splitting as large as 4500 Hz [17]. The ^{17}O splittings found in liquid crystals have been used to probe the phase structures, phase transitions and water orientations in heterogeneous systems [11–18], and should also be useful in study of hydration of biomolecules, if the liquid crystal phases are created by biomolecules. Theory has indicated that the five transitions in the quintet relax at three different relaxation rates [19,20]. However, direct application of the theory to the ^{17}O quintet has not been available.

In recent years the anisotropic environment offered by liquid crystals has aroused much attention in biomolecular NMR, because dipolar couplings can be partially recovered when proteins and nucleic acids are dissolved in the liquid crystalline solutions [21]. Filamentous bacteriophage, Pf1, as a user-friendly and tunable anisotropic

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medium, has been widely used in NMR for the purpose of slightly orientating NMR molecules [22,23]. Pf1 particles have been identified as single stranded long DNA molecules coated with polypeptides [24,25], which possess strong anisotropic magnetic susceptibility and can be easily aligned in a magnet. So far ^{2}H quadrupole splitting has served as a fast and sensitive indicator of the anisotropy. In our Pf1 phage solution that was going to be used for residual dipolar coupling measurement, ^{2}H NMR showed a quadrupolar coupling of 16 Hz at 25 °C, an indication for good alignment of the Pf1 phage particles. According to earlier NMR studies of water in heterogeneous systems, ^{17}O quadrupole splitting is usually approximately 6 times the ^{2}H splitting [11–18]. It was thus predicted that ^{17}O signal in the same system would be split to a quintet with a splitting approximately 100 Hz. This has been proved in our experiments. In this paper the partially resolved water ^{17}O quintet found in filamentous phage solution is reported. Quadrupolar relaxation theory is applied to the line shape analyses of the ^{17}O quintet. By simulation of the quintet, hydration number per nucleotide of the phage molecule has been evaluated.

2. Experimental

Filamentous phage Pf1 were prepared and purified according to the protocol described in Ref. [23]. *Pseudomonas aeruginosa* host, which were obtained from American Type Cell Collection (ATCC 25102), were grown at 37 °C in 50 ml LB-media (10 g tryptone, 5 g yeast extract and 10 g NaCl) in a 250 ml shaker flask to an OD_{600} of ~1.0. Then, 50–100 μl of re-suspended Pf1 (from ATCC 25102-B1) were used to infect the host cells, which were allowed to grow for a further 15 min. After inoculations in baffle flasks containing media, the cells were grown 14 h to stationary phase. The phage were harvested by centrifuging the host out of solution at 11 300×*g* for 45 min. Cell pellets were discarded and phage were precipitated from the supernatant by adding 60 g/l NaCl and 20 g/l of polyethyleneglycol. The phages were pelleted out of solution at 5 °C. Each pellet was re-suspended in 5 ml distilled water. Purification of phage was achieved using a KBr density gradient. Gradients were poured as block layers of 50, 42, 36, 28 and 20% KBr with the phage dissolved in the top layer. Ultracentrifugation (at 82 700×*g*) was then used for 24 h at 25 °C. The phage, which appeared as blue-gray band in the density gradient, was extracted with a syringe. Purified phages were then extensively dialyzed into 1.0 mM EDTA, 10 mM Tris pH 7.9. The final product of the Pf1 solution was 14 mg/ml in concentration, as determined by UV absorbance.

In order to introduce D_2O into the solution for NMR lock while keep the phage concentration unchanged, the volume of the solution was first reduced by ultracentrifugation at 6000 rev./min in a Beckman JA20 rotor at 15 °C for 2 h and then D_2O was added to reach the original volume. D_2O to H_2O ratio was approximately 1:9.

NMR experiments were conducted on a Bruker Avance 600 spectrometer with an inverse broadband probe tuned at 81.354 MHz for ^{17}O and 92.127 MHz for ^{2}H. For ^{17}O NMR, 4000 transients (90° excitation) were accumulated with 100 ppm sweep width, 125 ms acquisition time and 200 ms relaxation delay. For ^{2}H NMR, 8 scans gave spectra with good signal-to-noise ratio.

NMR line shape simulation was performed using Mathsoft Mathcad (version 2001). Input parameters were adjusted in a trial-and-error manner until reasonable line shapes were obtained.

3. Results and discussion

The ^{17}O experiments began at 25 °C. The acquired spectrum (data not shown) did not show a normal Lorentzian line shape. Instead, it seemed to be a spectrum overlapped with multiple transitions. By lowering the temperature down to 10 °C, the line width was reduced instead of increased. This was contrary to the normal phenomenon that lower temperatures usually lead to a broadened line shape because the molecular tumbling is slowed down. At 50 °C, a triplet was observed with a splitting approximately 100 Hz. At 70 °C, the splitting did not change much but the line shape clearly meant a partially resolved quintet. Fig. 1a shows the ^{17}O spectra at four temperatures. The line shapes were repeatable, i.e. after raising

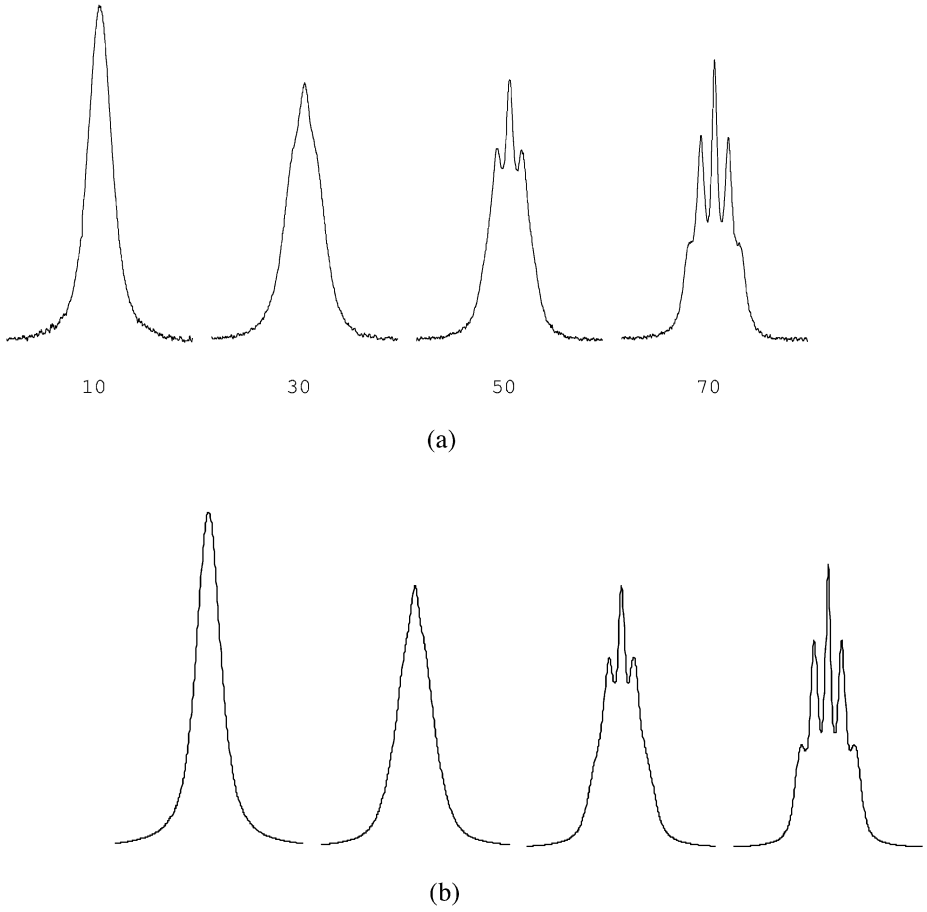

Fig. 1. (a) ^{17}O NMR spectra from a phage solution (90% H_2O/10% D_2O) at different temperatures (10, 30, 50 and 70 °C, as indicated in the figure). A Lorentzian line broadening factor of 10 Hz was used to smooth the spectra and the plot region for each spectrum is 1500 Hz. At 70 °C, the spectrum shows a partially resolved quintet. (b) Simulated spectra by taking into account the external EFG stemming from the Pf1 phage lattice. In simulation all spectra were assumed to consist of two components, the fast motion and the slow motion components, both being coupled to the external EFG with a Gaussian distribution. From 30 to 70 °C, the two components were assumed to undergo fast exchange, while at 10 °C, slow exchange was assumed.

and lowering the temperature for several times they appeared the same. But when the temperature went too high (80 °C and higher), the sample went wrong and precipitation occurred.

According to the 'sharp' selection rule of quantum mechanical transition [26], the quintet for a 5/2 spin should show an intensity ratio of 5:8:9:8:5. However, since the outer transitions generally relax with faster rates [19,20], it is not surprising to see that the intensity ratio of the quintet deviates from the ideal quantum mechanical ratio with the side transitions more broadened than the central transition. But when the relaxation theory was applied to quantitatively analyze the line shape, it was found that the relaxation alone was not able to fully account for the broadening of the side transitions. There could be other mechanisms behind the observed line shapes.

In quantitative interpretation of the ^{17}O NMR line shapes in Fig. 1a, site exchange between the bound and free states of the water molecules should be taken into account. Due to the rapid site

exchange, the relaxation rate for a specific transition p in the quintet is

$$R_{2,p} = R_{2b,p}x_b + R_{2f,p}(1-x_b) \quad (1)$$

where the subscripts b and f refer to bound and free states, respectively, and x_b is the fraction of the bound water. For ^{17}O in the bound state where the molecular motion is assumed to be within slow motion limit, previous studies reveal that the relaxation rates for the five transitions are not the same [19,20]. For convenience, we denote the central transition $(1/2 \leftrightarrow 1/2)$, the inner pair of side transitions $(\pm 1/2 \leftrightarrow \pm 3/2)$ and the outer pair of side transitions $(\pm 3/2 \leftrightarrow \pm 5/2)$ with 0, 1 and 2, respectively. Then we have the relaxation rates for the slow motion component

$$R_{2b,\,0} = K[8J_1(\omega_0) + 28J_2(2\omega_0)], \quad (2a)$$

$$R_{2b,1} = K[3J_0(0) + 18J_1(\omega_0) + 23J_2(2\omega_0)], \quad (2b)$$

$$R_{2b,2} = K[12J_0(0) + 24J_1(\omega_0) + 14J_2(2\omega_0)], \quad (2c)$$

where

$$K = (\pi^2/120)[(2I+3)/I^2(2I-1)]\chi^2(1+\eta^2/3). \quad (3)$$

Here χ is the local quadrupolar coupling constant in rigid solid, η is the asymmetry parameter, and

$$J_k(k\omega_0) = \tau_{cb}/(1+k^2\omega_0^2\tau_{cb}^2) \quad (4)$$

with $k = 0,1,2$. For ^{17}O in the free water molecules, which are assumed to be tumbling in the extreme narrow limit (though slightly anisotropically), the five transitions of ^{17}O relax with the same rate according to the isotropic formula

$$R_{2f} = 36K\tau_{cf} \quad (5)$$

All five transitions are assumed Lorentzian, with an average relaxation rate for each determined by Eq. (1).

Using the two-site exchange model, the spectra presented in Fig. 1a were simulated. In simulation

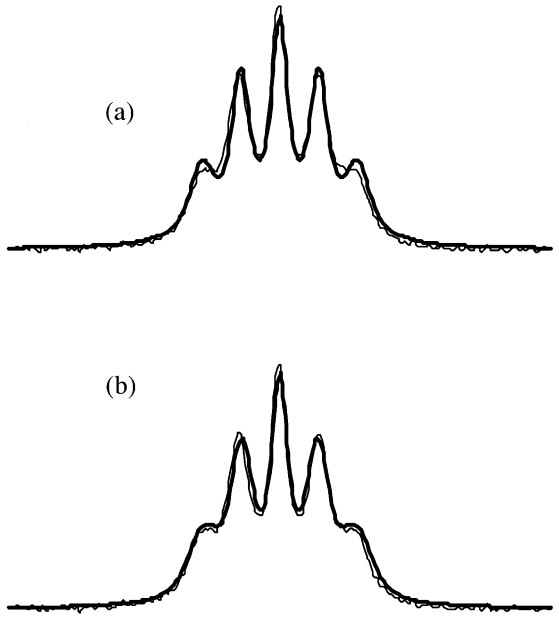

Fig. 2. Simulated ^{17}O spectra (in boldface) against the experimental spectra at 70 °C (in lean type), using the two-site exchange model without (a) and with (b) the external EFG considered. For (a), $\tau_{cf} = 3.1$ ps, $\tau_{cb} = 6.0$ ns and $x_b = 0.08\%$. For (b), $\tau_{cf} = 3.1$ ps, $\tau_{cb} = 3.98$ ns, $x_b = 0.045\%$ and 20 Hz Gaussian broadening factor was used. Note that the outermost side transitions in the simulation (a) are too narrow to fit the experimental spectra while this problem was effectively solved in (b) with the Gaussian broadening introduced to external EFG. No line broadening was applied to the experimental spectrum.

the integration ratio 5:8:9:8:5 was used as a constraint and the adjustable parameters included the correlation times τ_{cb} and τ_{cf}, the mole fraction of the bound water x_b, and the quadrupole splitting C_q. However, after numerous simulations no satisfactory results were obtained. When τ_{cf} was kept in the picosecond range, τ_{cb} in the nanosecond range and x_b lower than 0.08%, the side transitions in the simulated spectra were always too narrow to fit the experimental spectra. A typical simulated spectrum at 70 °C is presented in Fig. 2a in boldface, which is compared with the experimental spectrum in lean type. In simulation, $\tau_{cf} = 3.1$ ps, $\tau_{cb} = 6.0$ ns and $x_b = 0.08\%$. The bound water correlation time τ_{cb} (6 ns) was somewhat too large, because the bound water molecules at 70 °C

should move rather rapidly. The bound water fraction x_b (0.08%) was also too high, because it would correspond to a coordination number per nucleotide as large as 7 (see discussions below). Even with these parameters the spectrum was still not very well simulated. With τ_{cb} increased to 20 ns or x_b up to 0.12%, better line shape simulations had been obtained (data not shown). However, the simulated parameters were far from reality and were not acceptable.

It has long been noted that an oriented medium can produce an external electric field gradient (EFG) and that this external EFG also contributes to the observed residual quadrupole splitting [27]. For example, the residual quadrupolar coupling of ^{23}Na has been clearly referred to as the interaction between the quadrupole of ^{23}Na and the ordered environment [28]. The electric property of the phage is well known. A phage particle is a single stranded long DNA. Although the phage molecule is coated with polypeptides, its surface is still strongly negatively charged and a phage particle can be regarded as a charged rod possessing anisotropic susceptibility [22–25]. In the magnet, the charged rods are easily oriented parallel to the magnet, creating a useful anisotropic medium for biomolecular NMR study. The oriented and charged phage rods should produce strong EFG everywhere in the solution, and this has been experimentally verified. In Fig. 3 we compare two spectra, both being recorded at 70 °C. The upper trace is the spectrum from a sample containing 14 mg/ml phage (without D_2O) and approximately 2 mM Na^+, which was introduced along with EDTA. The lower trace is the spectrum when more NaCl was intentionally added to the solution (the concentration is estimated to be 100 mM). The addition of salt made the finely resolved quintet (upper trace) badly impaired to become a spectrum with much smaller quadrupole splitting estimated to be only 40 Hz (lower trace). The presence of more salt should not interfere with the alignment of the phage rods, nor should it seriously affect the motion of the water molecules. The decrease in the splitting upon addition of salt manifests a reduction of the strength of the external EFG, because the positively charged Na^+ should have strong interaction with the negatively charged

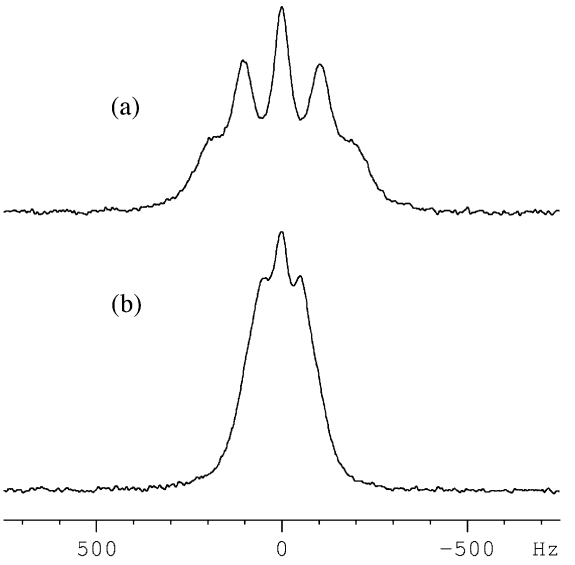

Fig. 3. Comparison of the ^{17}O spectra from the Pf1 solutions containing (a) 2 mM Na^+ and (b) 100 mM Na^+. Quadrupolar splitting is dramatically reduced upon addition of salt. The asymmetry appearing in (b) could probably be due to poor shimming (because D_2O was not contained in this sample) or small gas bubbles in the solution.

phage surface. Therefore, external EFG should be taken into account in the line shape analyses.

Phage particles do not distribute uniformly in the solution, as evidenced by electron micrograph [23]. Hence, the external EFG should not be homogeneous for all water molecules. The magnetic alignment, molecular diffusion and site exchange can, to some extent, improve the homogeneity of the external EFG, but at a given temperature the external EFG should disperse over a certain range. In other words, the quadrupole splitting C_q at a given temperature is not a constant, but distributes in the frequency space. For convenience, we assume a Gaussian function for C_q

$$G_{\pm p} = \exp[-(pa)^{-2}(C_q \pm pC_0)^2] \quad (6)$$

with a broadening constant a, a nominal splitting C_0 and $p = 1,2$. This distribution causes the two outermost side transitions doubly broadened as the two inner side transitions, but does not affect the

Table 1
Results of fitting the water ^{17}O NMR spectra in the Pf1 phage solution

T(°C)	Fast motion component		Slow motion component				x_b(%)	C_0(Hz)	a(Hz)
	τ_f(ps)	$R_{2f}(10^2\ s^{-1})$	τ_b(ns)	$R_{2b,0}(10^4\ s^{-1})$	$R_{2b,1}(10^4\ s^{-1})$	$R_{2b,2}(10^4\ s^{-1})$			
10[a]	9.12	4.96	–	–	–	–	–	40	25
30	6.37	3.47	11.7	0.73	6.40	22.5	0.046	79	38
50	4.04	2.19	6.92	1.20	5.00	14.7	0.043	100	31
70	3.10	1.64	3.98	1.90	4.71	10.5	0.045	105	20

[a] For simulation of the spectrum recorded at 10 °C, slow exchange model was applied and the parameters involving the slow motion component were unable to determine.

line width of the center transition. In other words, the center transition is still a Lorentzian, while each of the side transitions is a series of Lorentzians with Gaussian distributions. Explicitly, the side transitions are simulated using the Lorentzian function modified by a Gaussian function

$$S_{\pm p}(\omega, C_q) = R_{2,p} G_{\pm p} / [(R_{2,p})^2 + (\omega - C_q)^2] \qquad (7)$$

With these considerations, the simulation was much improved with a set of spectra very close to the experimental spectra as presented in Fig. 1b. The better simulation for the outermost side transitions can be clearly seen in Fig. 2b.

The simulated data for Fig. 1b are collected in Table 1. In simulations, we took the parameters $\chi = 6.68$ MHz and $\eta = 0.93$ from Ref. [19]. Although the external EFG contributes to the residual quadrupole coupling, it should not have any effects on the quadrupolar relaxation. The simulated correlation times for the fast motion component are in the picosecond range, in the same order of magnitude as in pure water. This is consistent with our assumption that the free water molecules in the phage solution can tumble freely. The correlation times for the slow motion component fall in the nanosecond range, in good agreement with the results found in cells and bovine serum albumin solutions (5–9 ns at 25 °C) [8]. The relaxation rates $R_{2b,1}$ and $R_{2b,2}$ steadily decrease as the temperature is increased, but the change of $R_{2b,0}$ is in reverse order. This is understandable, because $R_{2b,0}$ does not depend on $J(0)$ as can be seen in Eq. (2a).

The fast site-exchange model has been successfully applied to the analyses of the spectra at higher temperatures. However, for the spectrum at 10 °C, only slow exchange leads to good simulation. The lines of the slow motion component are too broadened to contribute to the line shape and the correlation time information about slow motion is not available.

The fraction of the bound water has been determined to be approximately 0.045%, from which the hydration number of the Pf1 phage particles can be readily obtained. The concentration of Pf1 is 14 mg/ml, approximately equal to 5.5 mM of nucleotide, since the content of DNA in phage is less than 12% by weight [24] and the average weight of deoxynucleotide in DNA is approximately 309. The water concentration is estimated to be 50 M and the concentration of the hydrated water should be approximately 22.5 mM. Then the hydration number per nucleotide is approximately 4.1.

The nominal coupling constant C_0 and the Gaussian decay factor a varies with temperature. While C_0 increases steadily with temperature, a does not. For the three cases with fast exchange, a decreases with temperature. A smaller a means a narrower distribution of EFG. This is reasonable because higher temperatures are advantageous for homogeneous external EFG, as will be explained below. For the spectrum at 10 °C, since the site exchange becomes slow on the NMR time scale and only unbound water molecules are considered, the distribution range becomes narrow again.

The contribution from the external EFG to the residual quadrupole splitting not only facilitates

the simulation, it can also be used to explain the temperature dependent splitting or the nominal coupling constant C_0. It has been proposed that the temperature dependent residual quadrupole splitting is due to the change in orientational probability distribution, and based on this proposition water orientations in liquid crystals at different temperatures have been studied [17,18]. However, it remains a puzzle why lower temperatures always tend to bring orientations favoring smaller quadrupole splitting. The presence of external EFG can give an alternative interpretation to the temperature dependence. Due to the rather high viscosity of the solution at lower temperatures, the alignment of the long phage particles is far from perfect. The disorder in the alignment of the phage rods and the difficulty in water diffusion can effectively reduce the strength of external EFG, so the residual quadrupole splitting is small. With the increase of the temperature, the phage particles are more stretched and the alignment is better. Water also diffuses more easily. As a result, the average external EFG is enhanced and the quadrupole splitting becomes larger. However, when the temperature is high enough, the phage particles tend to tumble, so the coupling cannot go higher. When the temperature is too high, the coating peptides would be detached and the phage rods will greatly change their electric property. Actually, when we increased the temperature to 90 °C, we obtained a ^{17}O spectrum without any fine structure (data not shown) and the phage solution eventually precipitated. It should be noted that fairly high temperatures cannot disentangle the entanglements of the phage filaments which where originally present in the solution. Hence warming and cooling the solution give repeatable line shapes.

It is not clear which contributes more to the residual quadrupole splitting, the external EFG or the internal EFG. Since the external EFG and the associated Gaussian distribution provide a reasonable explanation to the line shapes and the temperature effect of the residual quadrupole splitting, it can be estimated that the contribution from the external EFG could dominate over the internal contribution

4. Conclusion

We have observed ^{17}O NMR quintet from water in a filamentous phage solution. Temperature dependent line shapes have been analyzed and simulated. It turns out that the aligned phage filaments can produce effective electric field gradients that contribute to the residual quadrupole splitting and this contribution could not be neglected. The reorientation correlation times of free water are in the picosecond range, while the correlation times of bound water are nanoseconds. Through line shape analyses, the average hydration number per nucleotide of the phage DNA isdetermined to be 4.1.

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