Infrared spectroscopy on poly(dG)-poly(dC) DNA at low hydration

Hasanudin ABDURAKHMAN,[1,2,3,4] Kenji TAJIRI,[3] Hiroyuki YOKOI, [3] Noritaka KURODA,[3] Hiroshi MATSUI,[1,2] Takeshi YANAGIMACHI,[2] Masateru TANIGUCHI,[5] Tomoji KAWAI,[5] and Naoki TOYOTA[1,2]

[1]*Center for Interdisciplinary Research, Tohoku University, Sendai 980-8578*
[2]*Physics Department, Graduate School of Science, Tohoku University, Sendai 980-8578*
[3]*Department of Materials Science and Engineering, Graduate School of Science and Technology, Kumamoto University, Kumamoto 860-8555*
[4]*Faculty of Engineering, Tanjungpura University, Pontianak 78124, Indonesia*
[5]*The Institute of Scientific and Industrial Research, Osaka University, 8-1 Mihogaoka, Ibaraki, Osaka 567-0047*

Abstract

Infrared absorption measurements have been made on dry samples of poly(dG)-poly(dC) DNA at various relative humidity and temperatures. The water content, controlled by the relative humidity, reduces as temperature increases and reaches a very low value of ~ 0.1 wpn above 120 ^{o}C. This minimum water content is maintained when the samples are brought back to room temperature as long as they are kept at the relative humidity of ~0 %. The molecular vibrations, which characterize the backbone structure as well as the base stacking and pairing, indicate that our samples maintain an A-form double helical structure at all the values of water content. The disorder in the base stacking is observed as the result of the decrease of the water content. Additionally the denaturation appears at high temperatures above 100 ^{o}C, which reversibly disappears with decreasing temperature.

KEYWORDS: DNA, structure, infrared, water content, molecular vibrations

1. Introduction

The structure of DNA has been studied using various methods such as an X-ray diffraction, neutron scattering, and infrared spectroscopy. In the early stages of the structural studies, X-ray diffraction had been a unique method available for this purpose, while later, other methods have been applied to the analysis of the structure. One of the most important points obtained from those studies is the fact that DNA takes various conformations, and the conformational transition takes place due to several environmental factors[1,2)].

Fiberous samples have been used in early X-ray diffraction studies of DNA structure[1)]. This method has proved to be powerful for identifying a certain conformation, as well as the condition that favour particular conformations and that are responsible for introducing transitions between them. However, since the diffraction data of the DNA fiber are limitedto a resolution of 2 Å at best, such data hardly gives the structural information at an atomic resolution[1,3,4)]. Later, X-ray diffraction measurements have been performed also on the single crystals of oligonucleotides. Over the past two decades this method has provided the structural information at the atomic level, at least as far as atoms except hydrogen are concerned. However, unlike the situation within the fibers, the single crystal environment is rarely able to accommodate significant conformational transitions[3,4)].

As is well known, neutron scattering measurement is unique and powerful for studying biological systems. This is because the neutron scattering allow us to “see” the hydrogen atoms [5,6)]. This method, however, requires a sizable single crystal since in general its intensity is five orders of magnitude as low as X-ray intensity.

Finally to note, the infrared spectroscopy is also a powerful tool for providing structural information through the molecular vibrations. In the 500-2000 cm^{-1} region the infrared spectrum of a DNA gives about 40 well-defined absorption bands that appear at different positions with different relative intensities and dichroic ratios, depending on the conformation of DNA. Each spectral difference reflects the difference in the structure. A combination of the infrared spectroscopy with the X-ray diffraction have resulted in the sets of infrared marker bands of various conformations[7-10]. These marker bands make infrared spectroscopy very convenient for studying the structural properties of DNA.

The other advantage of infrared spectroscopy from the technical point of view is that there is no limitation for the sample size and one can work with native high molecular weight DNA, with DNA fragments obtained by an enzymatic or chemical cleavage, and with long stranded polynucleotides or short synthetic olygomers. In addition, it is a non-destructive technique that requires small amount of samples[10]. Many parameters such as the ionic strength and concentration of the counterion, pH, temperature, and hydration, can be easily monitored. Therefore, the infrared spectroscopy has been widely used to obtain the structural related properties as well as to study the structural change induced by the change in the environmental factors, such as relative humidity and temperature. For this reason the infrared spectroscopy has also been employed to explore the interaction of counterions with DNA[7].

In general, the conformation is strongly dependent on the relative humidity, and hence the water content. At high humidity a DNA fiber or film adopts the B-form at room temperature. As it is reduced to a certain threshold, the decrease in hydration leads to the transformation of the B-form into other conformations such as A or Z-form[1,9]. A

further decrease introduces a disordered structure. In addition, the denaturation can take place when the samples are heated up to ~90 ^{o}C. In the disordered structure, the hydrogen bonds connecting the bases are partially broken, resulting in a lack of the base stacking and pairing. In the denaturated state, on the other hand, most of the hydrogen bonds of the bases are broken, and DNA loses its double helical structure.

The infrared spectroscopy is convenient for studying the properties of water hydrated in DNA. Water molecules manifest themselves in the infrared absorption spectrum as several absorption bands arise from the OH stretching mode. Among them, the most intense band, which arises from the antisymmetric OH stretching mode, appears at around 3400 cm^{-1}[Ref. 11,12]. The intensity of this band is strongly dependent on the relative humidity as well as the water content. Therefore it has been widely used as the convenient measure of water content in DNA.

All three molecular subgroups in DNA (the heterocyclic bases, sugars, and diesterified phosphate groups) provide sites where water molecules can hydrate. The frequencies and intensities of the infrared bands associated with the various molecular subgroups are sensitive to environmental effects, particularly to the hydrogen bonding. The hydrogen bonding of water molecules with any molecular subgroups causes a shift in the vibration frequency. This shift, therefore, can be used for determining the hydration sites in a certain relative humidity range[11)].

Actually, the water content is not the only factor that determines the structure. In addition to water content, the structural properties are strongly dependent on the types[7,13-15)] and concentrations of counterions[16)], base sequence[1, 3,17)], and temperature[18)]. There have been many studies reporting the important roles of these factors. Those studies, however, focus mainly on the structural transition from one conformation to

another.

Up to date, the studies on the structural properties of dry DNA at low water content are very limited. The most recent work has been performed by Lee *et al*. on the natural DNA with sodium counterions using calorimetric and infrared spectroscopies[9]. They have found that, at room temperature, in the water content of 3.3 - 7.2 water per nucleotide (wpn) a structural change involving a slight disruption of the base stacking arises, followed by the denaturation process at higher temperature where the changes in the backbone structure occur. In case of natural DNA, the base sequence is random. Then, it is worth investigating the structural properties at low water content for differently sequenced DNA.

In this paper work we report on the results from infrared absorption measurements on dry poly(dG)-poly(dC) DNA with the regular base sequence at low water content. We are particularly interested in the structural stability of DNA at the state where the surrounding water is almost absent. Through this study, we intend to investigate the conditions to maintain the DNA structure at very low water content. A stable DNA structure at low water content might be important for further studies of DNA, particularly from the electrical conduction point of views.

2. Experimental

The samples of poly(dG)-Poly(dC) DNA with the typical molecular length of 1.7-2.9 μm was purchased from Amersham Bioscience Co. Ltd. The purification and collection were performed by using conventional method for an electroelution, phenol extraction, and ethanol precipitation. The processed samples then were diluted with deionized water (17.8 MΩ) to a concentration of 25 U (1 U = 50 ng/ml). The

concentration of sodium ions in the solutions was estimated as 1.2 x 10^3 ppm using an inductively coupled plasma spectroscopy. Finally, this solution was dried in a vacuum to obtain film-like solid samples.

A film for infrared absorption measurements was achieved by spreading the solid DNA on a BaF_4 crystal. The infrared absorption in the spectral range of 700-4000 cm^{-1} was measured using a FTIR spectrometer (Jasco FT/IR 410) equipped with a Cassegrain microscope. The samples were placed in a temperature-controllable optical cell (Linkam Th 6800). All the measurements were performed with a constant flow of dry nitrogen gas (500 ml/min.) into the sample chamber to keep the samples at low relative humidity, except for the first measurement at 30 ^oC.

3. Results and Discussion

Figure 1 shows the absorption spectra in the region of 2000-4000 cm^{-1} before and after the flow of dry nitrogen gas into the sample chamber at 30 ^oC and at higher temperatures. The spectrum is mainly featured by a broad band at 3400 cm^{-1}. Before the flow of nitrogen gas, the intensity of the band is quite strong. After 10 minutes of exposure to nitrogen gas, it abruptly decreases, and remains almost unchanged after the longer exposure to nitrogen gas. As temperature increases, the intensity gradually decreases up to 120 ^oC to become constant at higher temperature at 150 ^oC. It also remains constant as temperature is lowered back to 30 ^oC.

The band at 3400 cm^{-1} is assigned to the OH stretching mode of water molecules. This band is known to be strongly dependent on the relative humidity and has been used for estimating the water content in DNA[11,12]. It should be noted that several other vibrations such as NH and NH_2 stretching modes also appear in this frequency region

almost the same with the OH stretching mode[18,19]. Therefore, a careful identification of each vibration is necessary before using this band to estimate the water content.

To interpret on the spectrum carefully, we have measured the absorption spectrum of the guanine and cytosine powder, and the results are shown in Figs. 2 (a) and (b). The obtained spectra are in good agreement with previously reported results[19,20]. Each spectrum consists of several sharp peaks arises from NH and NH_2 stretching, observed below and above 3000 cm^{-1}, respectively. The two peaks of NH_2 stretching may correspond to the two main peaks in the spectrum of DNA shown in Fig.1. We have also measured the absorption of the bases at several temperatures up to 150 ^oC and observed no temperature dependence. The significant temperature dependence of the band at 3400 cm^{-1}, therefore, is considered as the property of the OH stretching of water molecules.

To separate the absorption band due to water from that of other origins, we subtracted each spectrum from the spectrum at 150 ^oC by assuming that, at this temperature, the remaining spectrum consists of the underlying absorption of DNA only, so that the subtracted spectrum can be regarded as the absorption spectrum of water. The results are shown in Fig. 3, and the intensity of the band as the function of humidity and temperature is shown in Fig. 4. The intensity is high at 30 ^oC before the flow of nitrogen (state I). It decreases after the 10 minute-flow, but remains unchanged after the longer exposure to nitrogen at the same temperature (state II).

As the temperature increases, the intensity decreases further, and becomes almost zero at 120 ^oC and higher temperatures (state III). This state remains as the temperature is lowered to 30 ^oC (state IV). It should be noted that the intensity recovers its initial value when the samples are exposed to room humidity.

Based on the previous reports[11], we consider the change of the intensity of the OH stretching band with the changing humidity and temperature is due to the change of the water content in our samples. In general, the water content of DNA can be measured by several methods, such as the so-called Karl Fisher titration and thermogravitometric[3]. However, we did not perform any measurements to check the exact value of the relative humidity and the water content of our samples. For a rough quantitative estimation, we use the formula given by Falk *et al.* [21]

$$n = 4.52(R - 0.86). \qquad (1)$$

Here R is the absorbance of the band at 3400 cm^{-1} without being subtracted with the spectrum at 150 ^{o}C, normalized by the absorbance of the band at 1240 cm^{-1}. Using the value of the absorbance plotted in Fig. 4 added by 0.43, which is the value of R at 150 ^{o}C and normalized with the absorbance of the band at 1240 cm^{-1}, we obtaine the water content for the sample prior to nitrogen gas exposure is 3-6 wpn.

After the flow of nitrogen gas, the water content is estimated to be ~1 wpn. Heating up the samples causes the remaining water molecules to evaporate. This evaporation is indicated by the further decrease in the intensity of the above-mentioned band as temperature increases. The water content in the sample after being heated above 120 ^{o}C is estimated to be ~ 0.1 wpn. Lee *et al.* have mentioned that they have completely removed water molecules from their samples by annealing them at 160 ^{o}C for 30 minutes[9]. Since the situation is very similar to our experiment, we believe that the value of water content of ~0.1 wpn above 120 ^{o}C is reliable.

The behavior of the band at around 1240 cm^{-1} in Fig. 5, which has been assigned to the antisymmetric PO_2^- stretching mode, also supports the above estimation on the water content. The frequency of this band is known to be strongly dependent on the

relative humidity[12,15]. In natural DNA at relative humidity of 70% and higher, it appears at ~1220 cm^{-1}, and almost linearly shifts to higher frequency as the relative humidity decreases. At 0 % humidity, it appears at ~1243 cm^{-1}. The exact frequency for each value of humidity varies depending on many factors such as the base sequence and counterion. Nevertheless, the relation between the humidity and frequency shift shows a similar trend for each kind of DNA.

Figure 5 shows the spectra in the region of 750-1800 cm^{-1}. At 30 ^{o}C before the flow of nitrogen in Fig. 5(a), we observe the antisymmetric PO_2^- stretching mode at 1240 cm^{-1}, which shifts to 1247 cm^{-1} after the flow of nitrogen (Fig.5(b)) and remains unchanged when the sample is brought to higher temperature. The frequency before the flow of nitrogen is very close to that of the sample at low hydration[11,12)], and the shift to higher frequency indicates that the water content of the samples decreases. This shift also indicates that the water molecules hydrate the phosphate backbone before being released.

It is now our concern to explore the structure of the present DNA sample at the state where water is almost absent. As described in Section I, the structural properties of DNA in the infrared spectrum is represented by several marker bands, which appear only for a certain conformation. In addition, the frequency of certain vibrations also shows the characteristics of a certain structure, such as the antisymmetric PO_2^- stretching, and the in-plane C=C or C=N stretching of the bases. Those bands, therefore, have been used as the indicator of the structural transformation as well as the structural disruption due to the change in the environment. The structural changes in our samples are easier to understand if we use each marked states in Fig. 4 as the guideline.

Figure 5(a) shows the spectrum measured at 30 ^{o}C prior to the exposure of the

samples to the nitrogen gas. This state of the samples corresponds to the state I in Fig. 4. The spectrum exhibits the characteristic of A-form with marker bands are observed at 806, 899, and 1182 cm^{-1}(Ref. 7). The absorption bands due to the symmetric and anti-symmetric stretching of the PO_2^- are observed at 1086, and 1240 cm^{-1}, respectively. The double bond (C=O and C=N) stretching mode of the bases, is observed at 1710 cm^{-1} which also reflect the characteristic of A-form [7,9,10].

As mentioned above, the water content of the samples significantly decreases after the exposure to nitrogen for 60 minutes, which corresponds to the state II in Fig. 4. However, this does not result in a significant change in the overall spectrum, particularly in the structural point of view. As shown in the spectrum of Fig. 5(b), the peak position and the shape of characteristic bands remain almost unchanged. Most of the bands are slightly enhanced, except the marker bands, which slightly diminish but remain clearly observable. The symmetric PO_2^- stretching mode at 1086 cm^{-1} also slightly diminishes and is broadened.

As the temperature increases (Fig. 5(c) and (d)), the intensity of each bands gradually decreases. These states correspond to the states II and III in Fig. 4. In particular, at 150 ^oC shown in Fig. 5(d), the A-form marker bands become barely observable, and the band at 1086 cm^{-1} representing the backbone structure is significantly reduced and broadened. The characteristic band of base stacking and pairing at 1710 cm^{-1} remains observable.

Figure 5(e) shows the spectrum measured at 30^oC after the sample is heated up to 150^oC. This state corresponds to the state IV in Fig. 4. The spectrum is very similar to that measured at the same temperature prior to the exposure to nitrogen gas, despite the difference in water content. In particular, the band at 1086 cm^{-1} becomes quite intense

and almost comparable to the intensity observed in the samples after the flow of nitrogen gas. The A-form marker bands, on the other hand, are very weak but remain observable.

As explained above, at 30 ^{o}C before the exposure to nitrogen gas (state I of Fig. 4) our samples take the A-form. After the exposure to nitrogen gas (state II), the samples maintain the A-form despite the decrease of their water content. A disorder, however, takes place, which is indicated by the diminishing of the marker bands. Lee *et al*. have observed that, in natural NaDNA at water content of 1 wpn, corresponding to the state II in the present work, the mode representing the base stacking and pairing at 1710 cm^{-1} disappears, while the symmetric PO_2^- stretching mode at 1086 cm^{-1} is broadened[9]. In contrast, both modes remain almost unchanged in our spectrum. This fact suggests that the degree of the disorder in our samples is much lower than that observed by Lee *et al*.

Our samples experience further disorder at high temperature (state II-III), which is indicated by the barely observable marker bands and the lowering intensity of the in-plane double band stretching of the bases. In addition, as shown in Fig. 6., the symmetric PO_2^- stretching mode is reduced and broadened with increasing temperature. This mode has been reported to be sensitive to the denaturation[9]. These facts indicate that, in addition to the slight disorder in base stacking and pairing, the denaturation also takes place at high temperatures above 100 ^{o}C.

In Fig. 7 we plot the intensity of the mode at 1086 cm^{-1} normalized at 1240 cm^{-1} as a function of temperature at different relative humidity together with the previously published data by Lee *et al*. The temperature dependence of the intensity of this band is very similar to the data of Lee *et al.* for the sample with 1 wpn. The change of the

intensity in this sample is small compared to the change of the intensity in the sample with 12 wpn,. Lee *et al*. have argued that this small change indicates that the samples are already in the denaturated state at room temperature because of the low water content. In contrast, as previously explained, our samples do not show any indication of denaturated state at room temperature.

Semenov *et al*. have identified that the intensity of the band at 1086 cm^{-1} is also strongly dependent on the water content[22]. Our data plotted in Fig. 7 is similar to their results. The intensity of this band decreases due to the flow of nitrogen at room temperature. The sharp change in the intensity of the same band in the sample with 12 wpn observed by Lee *et al*. could be attributed to the decrease of water content. They have mentioned that the samples are sealed during the whole measurement, but there may be no guarantee that water is not evaporated from the sample, particularly during the heating process.

In Fig. 5(e) the spectrum measured at 30 ^{o}C after the samples are heated to 150 ^{o}C reveals that the samples recover its A-form structure. This is indicated by the complete features for A-form, such as the marker bands, and the characteristic bands for the backbone and base pairs. It should be noted that this spectrum is measured at the state IV of Fig. 4, where the water content of the samples is ~0.1 wpn. It means that the denaturation at high temperature is mainly caused by the thermal energy, not by the lowering of the water content. On the other hand, a slight disorder observed at 30 ^{o}C after the samples are heated is attributed to the low water content.

The structural stability at very low water content observed in the present work is in contrast to the stating that a lack of water will result in a structural disruption of DNA. Our finding is similar to the previous observation on poly(dA)-poly(dT) DNA which

remain structurally stable at humidity of ~0%[23].

The counterion concentration is considered as the important factor for the structural stability of DNA. Water molecules and counterions have the same site where they have the highest affinity, i.e., the phosphate backbone. The water and counterion play the same role against the phosphate group; they screen the Coulomb repulsion between the negatively charged phosphate oxygens. At high hydration, this screening is mainly performed by water molecules. When the hydration is lowered, which corresponds to the lowering of water content, the counterions play the role of water molecules. In this case a high concentration of counterion, like the case of our samples, is required.

The structural stability of DNA at low hydration is very important for further exploration of electrical conductivity in DNA, a topic of controversy. Several theoretical calculations have predicted that water and counterions surrounding DNA influence not only its conformation, but also its electronic structure[24]. A particular configuration of DNA environment might induce a large shift in the location of Fermi level, which is relevant for the DC transport properties. Kino *et al*. have proposed that carriers can be doped to DNA by controlling the counterions[25]. Moreover, the doping states depend on whether counterions are hydrated or unhydrous. Particularly, a small amount of unhydrous Mg cations among the hydrated ones might act as impurities in poly(dG)-poly(dC) DNA. It is noteworthy that this doping state seems to be independent of the condition whether the DNA is in A- or B-form. The realization above mentioned theoretical proposal experimentally requires DNA with stable structure at any degree of hydration. In this context, our findings that the poly(dG)-poly(dC) DNA has a stable structure at very low hydration might be an important key toward it.

4. Conclusions

We have studied the properties of hydrated water and the effects of hydration on the structural properties in poly (dG)-poly (dC) DNA using the infrared spectroscopy. The water content of our samples at 30 ^{o}C and humidity of ~60 % is estimated to be 3-6 wpn. As humidity is lowered to 0 %, the water content decreases to 1 wpn. The water content reduces further to 0.1 wpn as temperature increases up to 120 ^{o}C, and that value remains at higher temperature. This state is maintained when the temperature is lowered to 30 ^{o}C while keeping the humidity at 0 %. Our samples maintain the A-form structure at all values of water content. The disorder in the base pairing is observed as the water content decreases. The high concentration of couterions is suggested as the origin of the stable structure despite its low water content. At high temperature, the disorder is accompanied by the denaturation, where the changes in the backbone occur. The denaturation is reversible against the temperature.

5. Acknowledgement

This work was supported by a Grant-in Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology (No. 16038205) and Grant for Research Program, from Center for Interdisciplinary Research, Tohoku University. The authors are grateful to Prof. M. Satake of the Institute of Development, Aging, and Cancer, Tohoku University for fruitful discussions.

References

1) W. Saenger: *Principles of Nucleic Acid Structure* (Springer-Verlag, New York 1984).
2) A. Ghosh, M. Bansal, Acta Cryst.: **D59** (2003) 620.
3) W. Fuller, T. Forsyth, A. Mahendrasingam: Phil. Trans. R. Soc. Lond. **B 359** (2004) 1237.
4) C.S. Delmonte, L.R.B. Mann: Current Science **85** (2003) 1564.
5) H.B. Stuhrmann: Rep. Prog. Phys. **67** (2004) 1073.
6) N. Niimura: Gendai Kagaku **396** (2003) 23 (in Japanese).
7) V.V. Andruschenko,. S.V. Kornilova, L.E. Kapinos, E.V. Hackl, V.L. Galkin, D.N. Grigoriev, Y.P. Blagoi: J. Mol. Struct. **408/409** (1997) 225.
8) J.A. Taboury, J. Liquier, E. Taillandier: Can. J. Chem., **63** (1985) 1904.
9) S.L. Lee, P.G. Debenedeetti, J.R. Errington, B.A. Pethica, D.J. Moore: J. Phys. Chem. **B 108** (2004) 3098.
10) J. Liquier, E. Taillandier, in H.H. Mantsch, D. Chapman (ed.): *Infrared spectroscopy of biomolecules*, (Wiley-Liss Inc., New York 1996) chap. 6, p. 131.
11) M. Falk, K.A. Hartman, R.C. Lord: J. Am. Chem. Soc. **85** (1963) 387.
12) P.B. Keller, K.A. Hartman: Spectrochim. Acta **42 A** (1986) 299.
13) S.V. Kornilova, P. Miskovsky, A. Tomkova, L.E. Kapinos, E.V. Hackl, V.V. Andruschenko, D.N. Grigoriev, Y.P. Blagoi: J. Mol. Struct. **408/409** (1997) 219.
14) T. Weidlich, S.M. Lindsay, A. Ruprecht: Phys. Rev. Lett., **61** (1988) 1674.
15) N. Lavalle, S.A. Lee, A. Ruprecht: Biopolymers **30** (1990) 877.
16) P.B. Keller, K.A. Hartman: Nucleic Acids Res. **14** (1986) 8167.
17) B. Wolf, S. Hanlon: Biochemistry **14** (1975) 1661.
18) G. Albiser, A. Lamiri, S. Premilat: Int. J. Biol. Macr. **28** (2001) 199.
19) B. Stuart: *Biological Application of Infrared Spectroscopy*, (John Wiley and Sons, New York, 1997).
20) E.R. Blout: M. Fields, J. Am. Chem. Soc. **72** (1950) 479.
21) M. Falk, A.G. Poole, C.G. Goymour: J. Can. Chem. Soc. **48** (1970) 1536
22) M. Semenov, T. Bolbukh, V. Maleev: J. Mol. Struct. **408/409** (1997) 213.
23) B. Sclavi, W.L. Peticolas, J.W. Powell: Biopolymers, **34** (1994) 1105.
24) R.G. Endres, D.L. Cox, R.R.P. Singh: Rev. Mod. Phys., **76** (2004) 195.
25) H. Kino, M. Tateno, M. Boero, J.A. Torres, T. Ohno, K. Terakura, H. Fukuyama: J. Phys. Soc. Jpn. **73** (2004) 2089.

Figure captions.

Figure 1. Absorption spectra in the region of 2000-4000 cm^{-1} at various humidity and temperatures.

Figure 2. Absorption spectrum of guanine (a) and cytosine (b) in the region of 2000-4000 cm^{-1} at 30 ^{o}C.

Figure 3. Absorption spectra in the region of 2000-4000 cm^{-1} at various humidity and temperatures after being subtracted by the spectrum at 150 ^{o}C.

Figure 4. Intensity of the absorption band due to the OH stretching mode as the function of exposure time to nitrogen gas and temperature. The states I and II are measured at 30 ^{o}C without and with the flow of nitrogen gas, respectively, the III is measured at 150 ^{o}C, and the state IV is measured at at 30 ^{o}C after the temperature is lowered from 150 ^{o}C.

Figure 5. Infrared absorption spectra in the region of 750-1800 cm^{-1} at 30 ^{o}C before (a) and after (b) the flow of nitrogen, we measure the high temperature spectra at 100 ^{o}C(c), 150 ^{o}C (d), and after the temperature is lowered back to 30 ^{o}C (e).

Figure 6. The symmetric PO_2^- stretching mode at various humidity and temperatures.

Figure 7. Hydration and temperature dependence of the intensity of the symmetric PO_2^- stretching mode.

Figure 1.

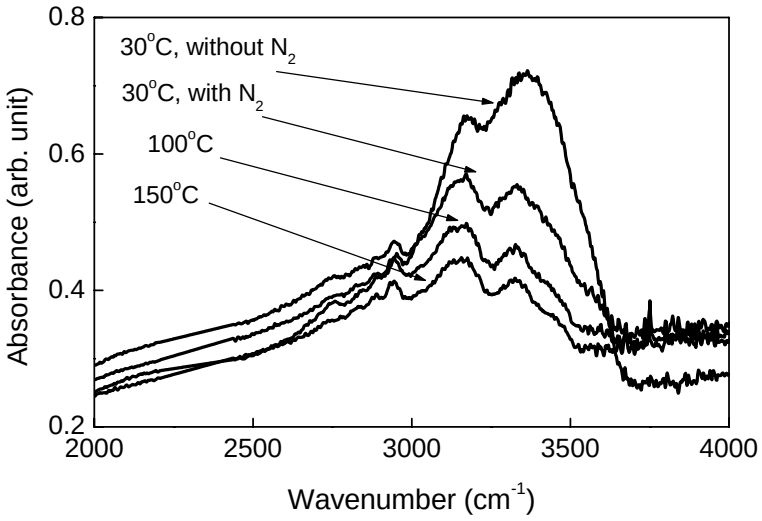

30°C, without N2
30°C, with N2
100°C
150°C
Absorbance (arb. unit)
Wavenumber (cm-1)
0.8
0.6
0.4
0.2
2000
2500
3000
3500
4000

Figure 2.

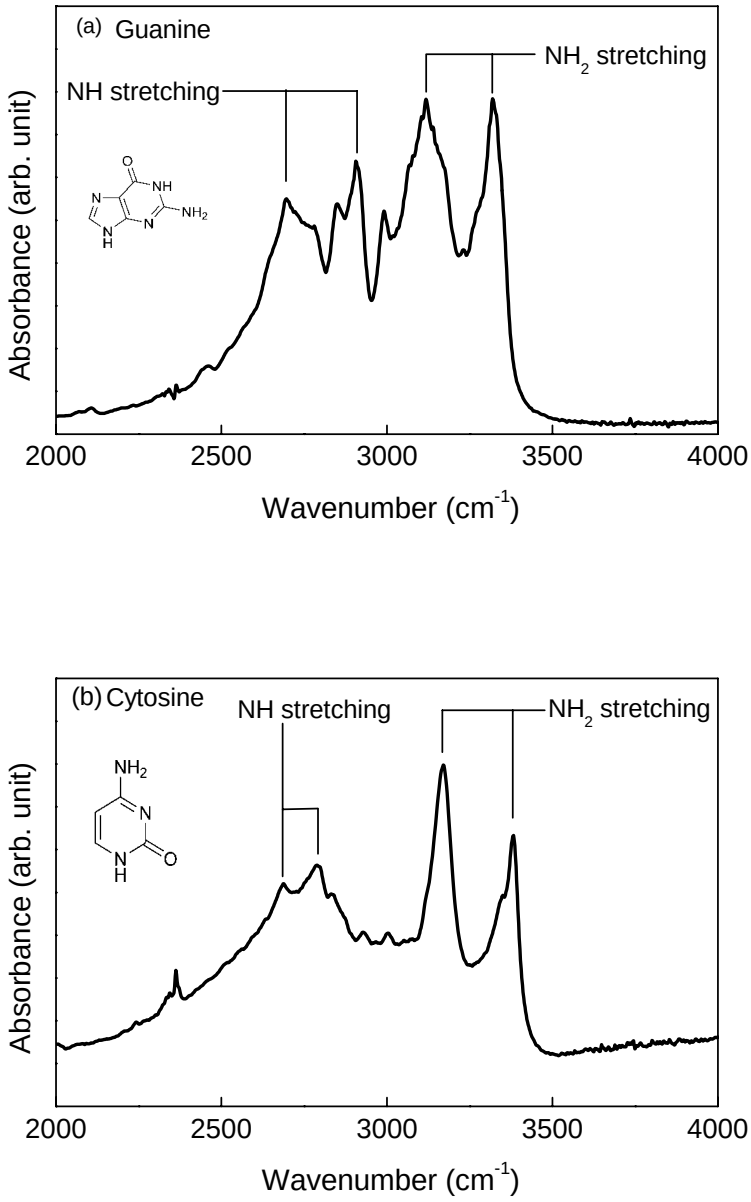

(a) Guanine
NH stretching
NH2 stretching
Absorbance (arb. unit)
2000
2500
3000
3500
4000
Wavenumber (cm-1)
(b) Cytosine
NH stretching
NH2 stretching
Absorbance (arb. unit)
2000
2500
3000
3500
4000
Wavenumber (cm-1)

Figure 3.

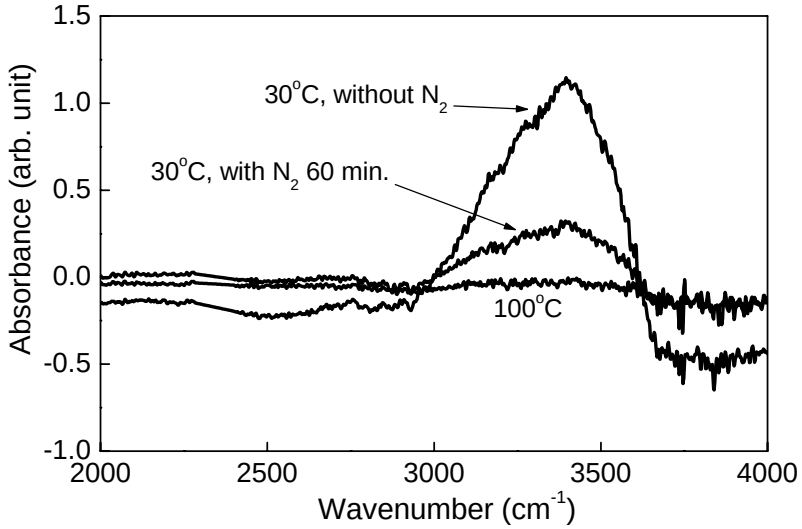

1.5
1.0
0.5
0.0
-0.5
-1.0
Absorbance (arb. unit)
2000
2500
3000
3500
4000
Wavenumber (cm-1)
30°C, without N2
30°C, with N2 60 min.
100°C

Figure 4.

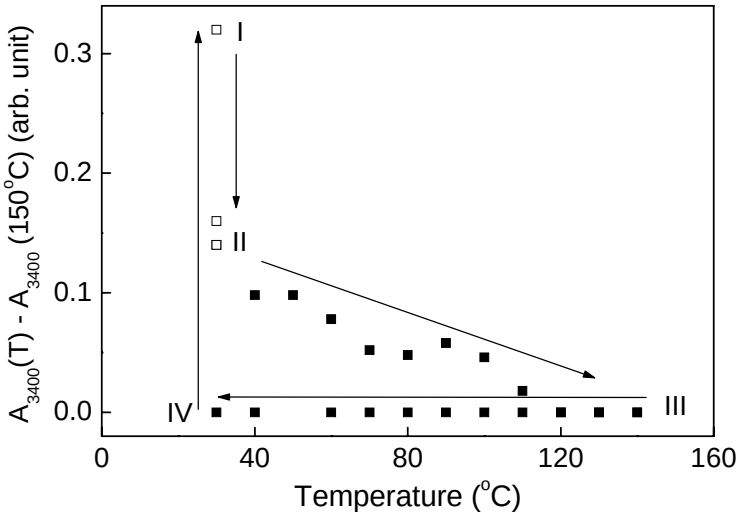

I
II
III
IV
0.0
0.1
0.2
0.3
0
40
80
120
160
Temperature (°C)
A3400(T) - A3400 (150°C) (arb. unit)

Figure 5.

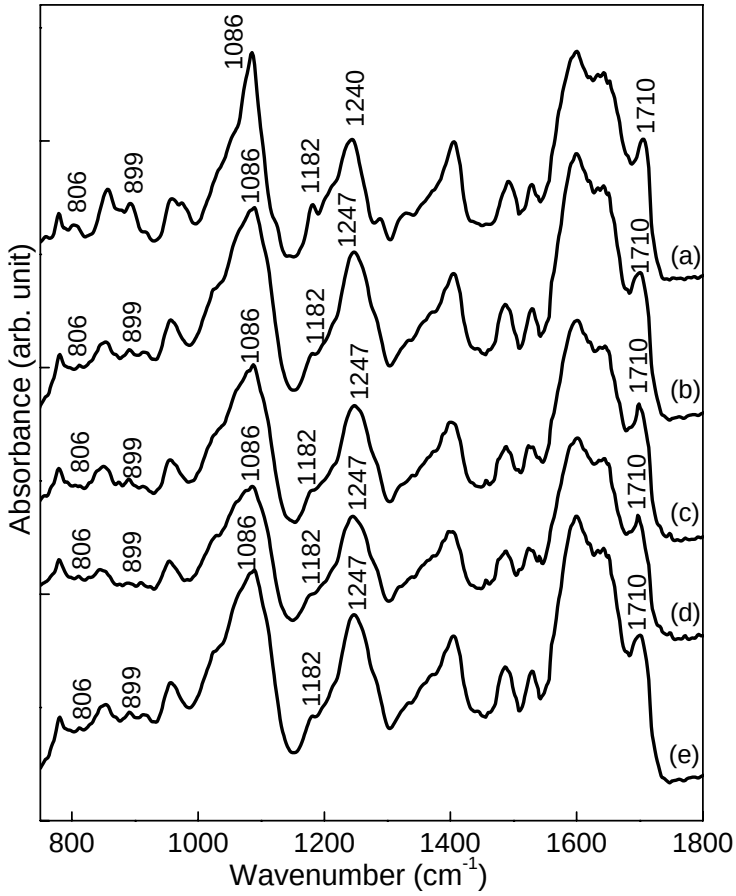

Absorbance (arb. unit)
Wavenumber (cm-1)
800
1000
1200
1400
1600
1800
806
899
1086
1182
1240
1247
1710
(a)
(b)
(c)
(d)
(e)

Figure 6.

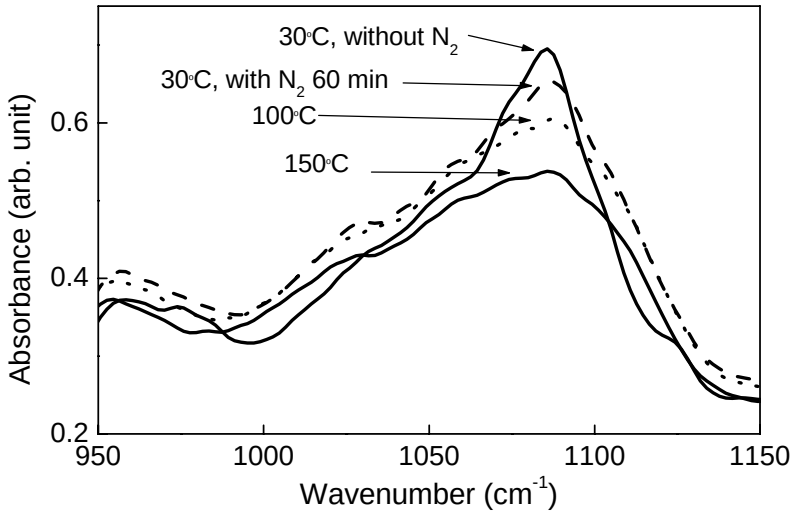
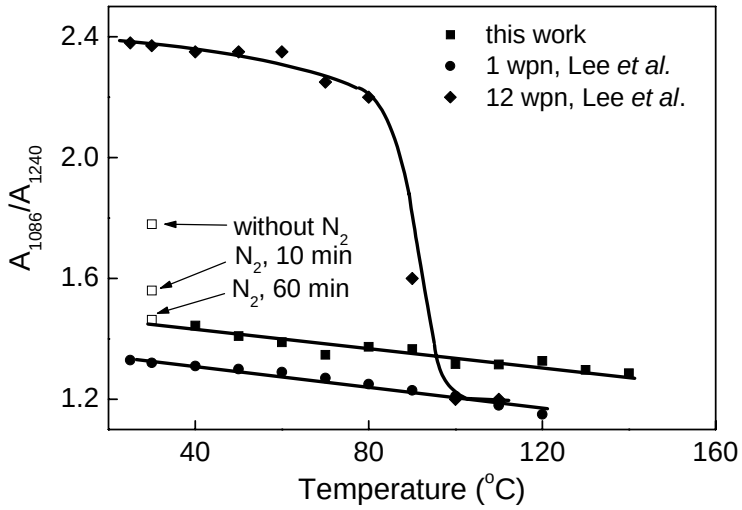

30°C, without N2
30°C, with N2 60 min
100°C
150°C
Absorbance (arb. unit)
0.2
0.4
0.6
950
1000
1050
1100
1150
Wavenumber (cm-1)

Figure 7.


2.4
2.0
1.6
1.2
A_{1086}/A_{1240}
40
80
120
160
Temperature (^{o}C)
this work
1 wpn, Lee *et al.*
12 wpn, Lee *et al*.
without N_2
N_2, 10 min
N_2, 60 min