THz Absorption of Adenine Base in Single-stranded Deoxyribonucleic Acid

Marina Komatsu[1], Yoshimichi Ohki[1, 2], Maya Mizuno[3]
[1]Dept. Electr. Eng. Bioscience, Waseda University, Shinjuku, Tokyo, 169-8555, Japan
[2]Res. Inst. Mater. Sci. Tech, Waseda University, Shinjuku, Tokyo, 169-8555, Japan
[3] National Institute of Information and Communications Technology, Koganei, Tokyo, 184-8795 Japan

***Abstract*—Absorption spectra were measured in a frequency range from 3 to 18 THz for various single-stranded deoxyribonucleic acids (DNAs) containing adenine as their bases. Nine absorption components in total, most of which can be assigned to either phosphoric acid or adenine, appear in the range observed. From the dependence of each absorption on the number of nucleotides in one DNA, the integrated molar absorption coefficient per nucleotide with adenine was estimated.**

I. INTRODUCTION

Deoxyribonucleic acid (DNA) is a basic element of all the living organs. It is a kind of polymer having a repeated structure of nucleotide composed of a deoxyribose, a phosphate, and a base. There are four different kinds of bases; adenine (A), guanine (G), cytosine (C), and thymine (T). Clarification of the structure of DNA has been of prime importance in medical and biological fields. In addition, a recent research trend that treats DNA as a new polymer material has enhanced the importance of the analysis of DNA. Therefore, in the present research, we try to correlate the number of bases in single-stranded DNA with absorption spectra obtained in a THz range as a first trial of THz spectroscopic analysis of DNA.

II. EXPERIMENTAL

The samples are single-stranded DNAs. The sample name consists of the letter A, G, C, or T that stands for the base and the number of constituent nucleotides. For DNA with A as their bases, samples with 24 to 100 nucleotides, denoted by A24 to A100, were used. For comparison, samples T80 consisting of 80 nucleotides with only T as their bases, G32 consisting of 32 nucleotides with only G, and C40 consisting of 40 nucleotides with only C were also used.

We first made an aqueous solution of DNA with a designated molar concentration. After it was dripped on a diamond substrate with a thickness of 0.3 mm, it was dried naturally. We measured THz absorption spectra of these naturally dried DNAs in a wave number range from 100 to 600 cm^{-1} (3 to 18 THz) using a Fourier-transform spectrometer (Jasco, VIR-F) at room temperature in air.

III. RESULTS AND DISCUSSION

Fig. 1 shows absorption spectra in a wave number range from 100 to 600 cm^{-1} measured for A100, T80, G32, and C40. Apparent small absorbance of G32 compared to the others is simply due to the fact that G32 has a low concentration of DNA with a small number of nucleotides. Each vertical bar shows ±one standard deviation of three measurements. At first glance, regardless of the type of base, broad and rather strong absorption can be recognized in a range from 100 to 300 cm^{-1} and at around 540 cm^{-1}. In addition, characteristic absorption features are seen at around 243, 335, 395, 450, and 480 cm^{-1} in A100, while at around 350, 420, and 490 cm^{-1} in T80.

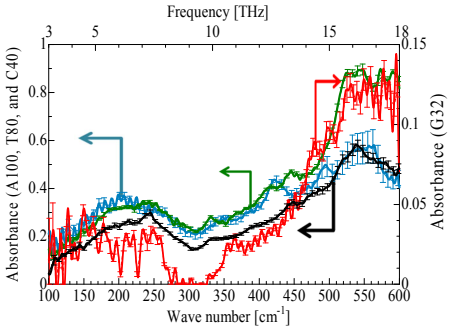


Fig. 1. Spectra of absorbance of A100 (—), T80 (—), G32 (—), and C40 (—) from 100 to 600 cm^{-1}.

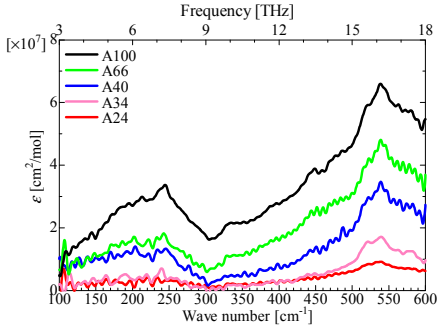


Fig. 2. (a) Molar absorption coefficients ε of single-stranded DNAs with 24, 34, 40, 66, and 100 nucleotides with A, denoted by A24, A34, A40, A66, and A100, respectively.

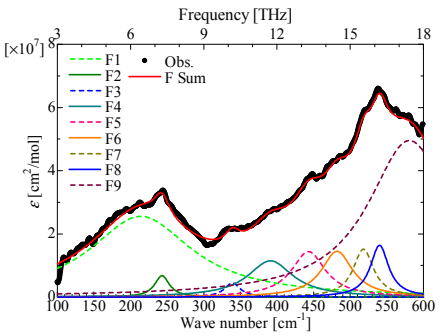
Moreover, C40 exhibits similar features at around 245, 330, 415, and 445 cm^{-1}, while G32 exhibits absorption at around 360 cm^{-1}. The wave numbers of the peaks of all these characteristic absorption features are different. Therefore, it is reasonable to assume that these absorption peaks are due to different respective bases in DNA samples. In this paper, we focus on DNAs containing A as their bases and discuss the dependence of THz absorption on the number of nucleotides.

First, we calculated molar absorption coefficients ε for all the samples containing A using the following equation:

$$\varepsilon = A/(\rho d) = A\pi r^2/M \ . \qquad (1)$$

Here, A is the absorbance or the spectral intensity of each sample shown in Fig. 1 and ρ is the molar concentration of the sample with a right cylindrical shape, while d and r are its thickness and radius and M is the amount of DNA in it denoted by the unit of mole. Fig. 2 shows ε calculated for DNA samples A24 to A100 consisting of 24, 34, 40, 66, and 100 nucleotides with A. The spectral intensity shows a monotonic increase with the increase in the number of nucleotides in the sample.

We tried to separate each spectrum shown in Fig. 2 in order to discuss the dependence of the intensity of each absorption on the number of nucleotides. As mentioned above, apparently, there are absorption peaks at around 100-300, 243, 335, 395, 450, 480, and 540 cm^{-1}. Moreover, if we watch the absorption at around 540 cm^{-1} carefully, it has two shoulders at 520 and 580 cm^{-1}. On the basis of such observation, the spectrum of A100



	Peak wave number [cm^{-1}]
F1	214
F2	243
F3	339
F4	391
F5	443
F6	482
F7	518
F8	540
F9	581

Fig. 3. Spectrum of molar absorption coefficient ε of DNA with 100 nucleotides having only A (A100), fitted to nine Lorentzian curves F1 to F9.

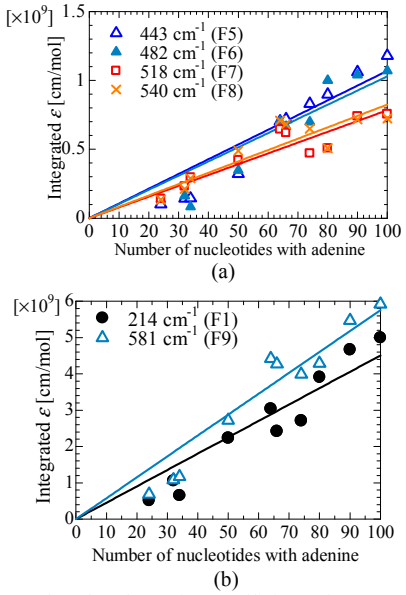


Fig. 4. Integrated molar absorption coefficient of DNA with only A as its base, as a function of the number of nucleotides.

was separated to nine Lorentzian peaks. Here, their intensities, positions, and widths were shifted from their respective initial values. Fig. 3 and the table beside it show the nine Lorentzian-fitted peaks, F1 to F9, with their wave numbers and their sum.

In order to examine the validity of the peak separation, the peak wave numbers are compared with those observed in an aqueous solution of phosphoric acid, A (adenine) in gaseous and crystalline forms, and other DNA components, reported in literature [1-9]. As a results, the absorption at 243 cm^{-1} (F2) shown in Fig. 1 can be assigned to "buttefly" like out-of-plane bending of a purine molecule [1]. Similarly, the absorption peaks at 339 (F3), 391 (F4), 482 (F6), 518 (F7), and 540 (F8) cm^{-1} are due to C(6)-NH_2 bending [1], NH_2 torsion [1, 2], NH_2 twisting [3], NH wagging [2], and in-plane skeletal deformation vibration [1], respectively. The absorption F5 at 443 cm^{-1} would also be assigned to A (adenine), since it has been reported that a similar peak appears in rebose if it forms adenosine by being combined with A [6, 7]. However, it is true that C40 also exhibits absorption at around 445 cm^{-1} as shown in Fig. 1, which is in the vicinity of F5. As for the absorption F9 at 581 cm^{-1}, it has been reported that A exhibits absorption at 563 and 576 cm^{-1} [3]. Phosphoric acid is known to show absorption at 570 cm^{-1} [8]. Because of the proximity of wave number to these absorption peaks, F9 may include the absorption of both A and phosphoric acid. Lastly, the absorption F1 ranging from 100 to 300 cm^{-1} seems to be due to a phosphate-water complex in nucleotides [9]. Therefore, the validity of Lorentzian fitting to the nine component curves shown in Fig. 3 has been confirmed. Moreover, bases T, G, and C do not have peaks F2 to F4 or F6 to F8 that are assigned as mentioned above. This in turn means that the peaks F2 to F4 and F6 to F8 are due to absorption characteristic to A, and that F1 would appear irrelevantly to the type of base in DNA.

As a next step, the six component absorption peaks, F1 and F5 to F9, were numerically integrated to calculate the integrated molar absorption coefficients with a unit of cm/mol. Note that F2, F3, and F4 were discarded, since their intensities are very small and not reliable, especially when the number of nucleotides is less than 80. Fig. 4 shows the estimated coefficients of the six absorption components. By drawing straight lines connecting the coordinate origin and data points, the integrated molar absorption coefficient per nucleotide can be calculated to be 1.0×10^7, 7.8×10^6 and 8.2×10^6 cm/mol for the three absorption components F6 to F8 at 482, 518, and 540 cm^{-1}, which are assigned to A. For components F1 at 214 cm^{-1}, F5 at 443 cm^{-1}, and F9 at 581 cm^{-1}, the integrated molar absorption coefficient per nucleotide is calculated to be 4.5×10^7, 1.1×10^7, and 5.8×10^7 cm/mol, respectively. Using these coefficients, we can estimate the number of nucleotides with A in single-stranded DNA with only A as their bases by THz spectroscopy.

IV. Acknowledgment

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