

THz spectroscopic characterization of biomolecule/water systems by compact sensor chips

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We demonstrate the terahertz (THz) spectroscopic performance of highly integrated sensor chips based on microstrip lines by measuring biomolecule/water systems. The concentration resolution of the present chips reaches down to 0.05 g/ml. We have confirmed that the number of bound water molecules per biomolecule can be obtained with precision using solid state transmission lines. The chips are highly suitable for the inspection of small amounts of specimen and for the application to a wide range of water rich materials. Our method may therefore be a good candidate for a simple liquid sensor working in the THz regime. © 2006 American Institute of Physics.

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Terahertz dielectric spectroscopy (THz-DS) is an indispensable tool for sensing dynamical responses such as intermolecular vibrations and structural information in specimens as recently shown for the case of a micelle.^{1,2} These unique features of the standard THz-DS are highly suitable for the application to improved security checks or quality control of industrial products. But the present THz-DS systems are insufficient for a more convenient sensing due to the rather large area of THz wave propagation. Therefore it is very important to develop a sensor incorporating a reduced area of propagation.

One of the best candidates for such integrated THz-DS systems are sensor chips using solid state transmission lines, which are now being developed.^{3–5} Recently we have realized compact sensor chips making use of the standard THz-DS.⁵ They are based on embedded thin-film microstrip lines and possess two remarkable features.⁵ One is the optical access from the backside of the chip, which is necessary for the convenient generation and detection of THz waves. The other is the controllability of spectroscopic sensitivity by the adjustment of the thickness of a polymer cover layer, which greatly simplifies the measurement of polar materials. By permitting the use of optical fiber for the light pulse delivery, the present chip promises a wide range of future applications, e.g., as a remote sensor head, which cannot be realized by other methods.

In this letter, we report the results obtained from biomolecule/water systems to demonstrate the performance of our chip in sensing the structural information of solutions. The structural information in this case is the number of the bound water molecules per biomolecule, N_B . That this information can be derived from sensing with THz waves has already been demonstrated by free-space transmission THz spectroscopy of a protein/water system.⁶ A precise analysis has been performed for several kinds of protein/water systems by microwave (0.2–20 GHz) dielectric measurements.⁷ In order to compare the spectroscopic performance of our device with these methods, we present results for the protein

bovine serum albumin/water (BSA/W) which has been well investigated.⁷ In addition, we have measured an amino acid L-arginine/water (L-Arg/W) system to shed light on the role of the hydrophilic amino acids of the protein.

The detailed device structure has been described elsewhere.⁵ THz waves are generated and detected by photoconductive (PC) switches made of low-temperature-grown GaAs films excited by a Ti-sapphire short pulse laser. The distance of 1 mm between the PC switches is the interaction length of the THz waves with the specimen. The spectral range of the present chips is from 50 GHz to 1 THz. The globulin-free BSA (GF-BSA, purity: 98%), fatty-acid-free BSA (FF-BSA, purity: 98%) and L-Arg were purchased in the form of powders from NACALAI TESQUE, INC. Specimens were prepared by dissolving the reagents with no further purifications into the pure water. The concentration c was controlled up to near the saturation level. A volume of about 0.14 cm³ of the solution was pipetted into a polyethylene specimen cell in which the whole sensing region, including the PC gaps, was contained.

In the inset of Fig. 1, output THz wave forms of GF-BSA/W are given at several concentrations c . The signal denoted as reference represents the wave form in the unloaded chip. A systematic reduction in the attenuation of the signal is observed with increasing c . To assess the concentration resolution, the peak intensities of THz wave forms normalized at $c=0$ are plotted as a function of c in Fig. 1 for all systems. Almost linear c dependencies are confirmed in two BSA/W systems within experimental uncertainties, while slight departure from linearity seems to occur in L-Arg/W. These results indicate a concentration resolution of about 0.05 g/ml for our chip.

The effective amplitude absorption coefficient α_{eff} of a specimen is defined as

$$\alpha_{\text{eff}}(f) = -\frac{1}{L} \ln \left(\frac{E_s(f)}{E_{\text{ref}}(f)} \right), \quad (1)$$

where L is the sensing length of 1 mm and $E_s(f)$ [$E_{\text{ref}}(f)$] is the Fourier transformed amplitude of the output THz wave form for the specimen (reference). Figures 2(a) and 2(b) show the $\alpha_{\text{eff}}(f)$ determined from the output wave forms for GF-BSA/W and L-Arg/W, respectively. The slightly different

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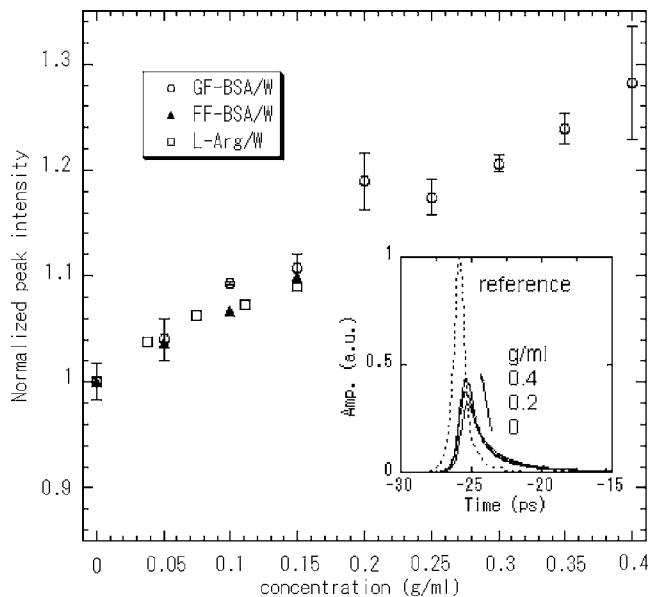


FIG. 1. Concentration dependence of peak intensities of THz wave forms normalized at $c=0$. Only error bars of GF-BSA/W are shown for clarity. Errors of the other systems are close to that of GF-BSA/W. The inset shows the THz wave forms for GF-BSA/W.

$\alpha_{\text{eff},w}(f)$ of water ($c=0$) in Figs. 2(a) and 2(b) originate from the fact that the chip used for GF-BSA/W is not identical to that for L-Arg/W. The $\alpha_{\text{eff},w}$ of water ($c=0$) is smaller than the reported value ($\sim 110 \text{ cm}^{-1}$ at 1 THz)^{8,9} due to the weakened interaction with the THz waves. That $\alpha_{\text{eff},w}(f)$ correctly represents the dielectric response of pure water can be confirmed by rescaling the reported value⁵ accordingly. The systematic decrease in α_{eff} with increasing c over the measured frequency range indicates the decreasing number of bulk water molecules.

The reduction ratios $r_{s/w} = \alpha_{\text{eff}}(f) / \alpha_{\text{eff},w}(f)$ are shown in Fig. 3 for GF-BSA/W and L-Arg/W. The appearance of frequency dependencies in $r_{s/w}$ implies a violation of the Lambert-Beer law, which predicts no frequency dependence. Supposing that the $r_{s/w}$ are attributed only to the subtraction

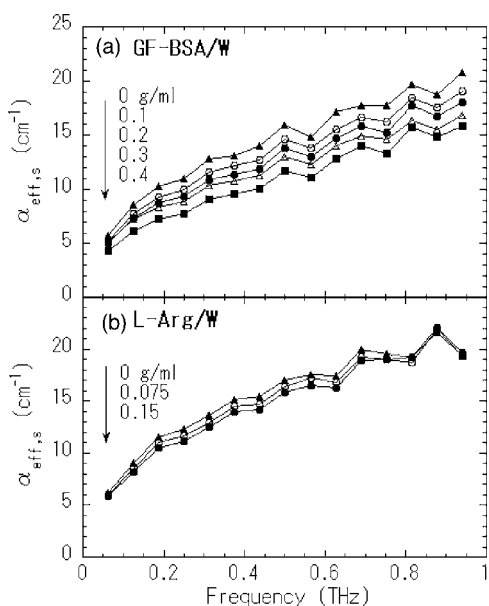


FIG. 2. Frequency dependence of α_{eff} of (a) GF-BSA/W and (b) L-Arg/W. The solid lines have been inserted as guides to the eyes.

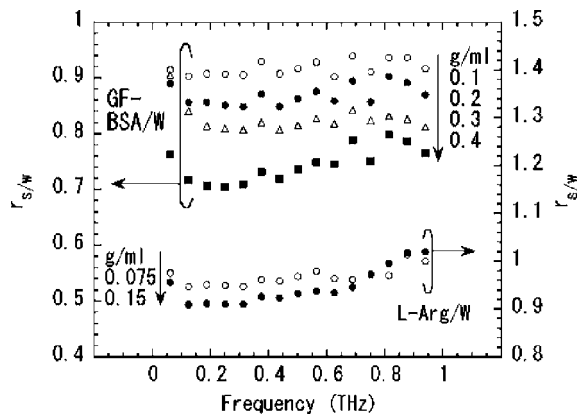


FIG. 3. Frequency dependence of $r_{s/w}$ for GF-BSA/W and L-Arg/W at several concentrations.

of bound water molecules from the bulk water molecules, the number of bound water molecules per biomolecule N_B can be estimated by

$$N_B = \frac{M_s(1 - r_{s/w})}{M_{\text{H}_2\text{O}}c}, \tag{2}$$

where M_s and $M_{\text{H}_2\text{O}}$ are the molecular weight of BSA (66430.3)¹⁰ or L-Arg (174.2) and that of the water, respectively. The values of N_B are shown in Fig. 4 for GF-BSA/W and L-Arg/W. Excluding the results at lower c primarily due to their rather low accuracy, the values of N_B including that of FF-BSA/W (not shown) are determined to be 2500 ± 300 (GF-BSA), 2400 ± 400 (FF-BSA) and 6 ± 1 (L-Arg), if we use the results obtained at 0.2–0.3 THz. Each N_B steadily decreases down to 2100 ± 300 , 1750 ± 500 and 1 ± 3 around 0.8–0.9 THz. The difference between the N_B of GF-BSA/W and that of FF-BSA/W cannot be clearly discerned. Taking into account that BSA contains 583 amino acid residues,¹⁰ the number of water molecules bound by an individual amino acid residue in a protein can be deduced from our results.

Returning to the reduction ratios $r_{s/w}$, the frequency dependence of $r_{s/w}$ is prominent for frequencies below 0.1 THz or above 0.4 THz. This prominence tends to be especially large at higher c . There exist two possible origins: instrumental problems and the specimens themselves. As an instrumental aspect, we first discuss the reproducibility of $\alpha_{\text{eff}}(f)$.

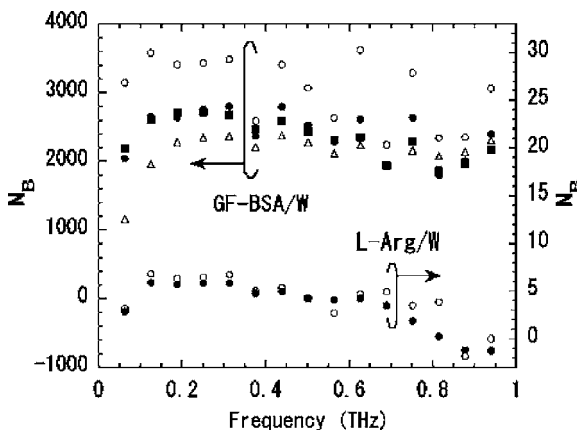


FIG. 4. Frequency dependence of N_B for GF-BSA/W and L-Arg/W at several concentrations. Marks in the figure represent the same concentrations as in Fig. 3.

Separately obtained $\alpha_{\text{eff},w}(f)$ by the same chip show errors of 0.5% and 1% above and below 0.1 THz, respectively. Hence the frequency dependence is obviously larger than this error. Effects of frequency dependent dielectric and/or radiation losses determined by elements other than the specimens might be another possible source of instrumental problem. This case could be induced by a redistribution of the THz electric field in the chip with the change of c . Such an effect is conjectured to be subtle, however, because the refractive index of the specimen would not considerably differ from that of the water, especially at higher frequency. For the low frequency (<0.1 THz) range, we must consider near field effects on transmission properties. In the frequency domain, the sensing length of the present chips (1 mm) corresponds to about 0.2 THz, where the effective refractive index of the transmission line for the unloaded chip (~ 1.65)⁵ is taken into account. The latter two effects cannot be evaluated precisely due to the lack of reliable expressions describing these features in the THz regime. On the other hand, as regards the nature of specimen, it is plausible to assume an influence of interactions between the solutes and/or between the solute and the water which is not considered in the Lambert-Beer law. This would severely affect the $r_{s/w}$ at higher c , which is consistent with the results in $r_{s/w}$ exhibiting the rather large frequency dependencies in the concentrated specimens. Although an expression for the dielectric constants established for solute/water systems at higher c ¹¹ can be employed for the analysis, one needs the absolute value of the complex dielectric constants of the solute/water and the solute beforehand. An analysis along this line therefore requires further study.

Finally, the comparison of spectroscopic performance of our method and other methods is briefly discussed. In BSA/W, the value of N_B obtained by our chips is roughly close to that obtained by the microwave dielectric measurement (~ 1300).⁷ Recently the hydration number of L-Arg in water has been found to be about 14 by THz attenuated-total reflection (ATR),¹² which is roughly consistent with our result (~ 5 at 0.2 THz). These results indicate that our chips work well in sensing the N_B of biomolecule/water systems. The sensitivity of the microwave dielectric measurement^{7,13} (10^{-2} or 10^{-4} g/ml) is still higher, but that of our chips can be improved by optimizing the strip-line design and by using lower noise instruments. It should also be noted that our chips would provide additional THz dynamical information which cannot be obtained by microwave spectroscopy. In

contrast to the free-space transmission THz spectroscopy, our chips can be easily applied to water rich specimens. Recent progress in THz-ATR⁹ should provide the dielectric constants of water rich specimens. Therefore our chips are possible practical liquid sensors to be calibrated by the ATR measurement results. At the present stage, the sensitivity of our chips seems to be comparable with that of the ATR measurements.

In summary, the THz spectroscopic performance of our recently developed sensor chips based on microstrip lines has been examined by measurements of biomolecule/water systems. The $r_{s,w}$ show a violation of the Lambert-Beer law, which may be due to instrumental problems and/or some kind of interaction in the specimen. Although the N_B are tentatively estimated, they should be confirmed by using the results of THz-ATR spectroscopy. The highly integrated spectroscopic chips are easily applicable to water rich systems, and may therefore be useful as liquid sensors working in the THz regime.

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