Terahertz Spectroscopic Study of Ion Effects on Protein Hydration

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***Abstract*—Effects of salts on the dynamical properties of hydration water of protein in aqueous solutions are studied using high-precision terahertz spectroscopy. Kosmotropic anions in the well-known Hofmeister series are found to decrease hydration water, and chaotropic anions increase it. This result is consistent with a theoretical model which describes the ion effects on hydration of proteins in terms of the hydrogen-bonding network around the protein.**

I. Introduction and Background

CONTROLING solubility, folding, and stability of proteins has been an important subject in protein engineering, pharmaceutics, and food industry. It has been known that the ability of anions to precipitate proteins and also anion effect on the stability of proteins are ordered in the Hofmeister series:

$$SO_4^{2-} > H_2PO_4^{-} > Cl^{-} > NO_3^{-} > SCN^{-}.$$

The left-side anions are called kosmotropes (structure makers), and the right-side anions chaotropes (structure breakers). The microscopic mechanism underlying the Hofmeister series has been discussed, and hydration of protein molecules is expected to play the critical role although clear experimental demonstration is still awaited.

Terahertz spectroscopy is a powerful tool clarifying the picosecond dynamics of water molecules, and has been used for the study of hydration of various molecules in aqueous solutions. Since hydration water is usually much less mobile than that of bulk water, leading to reduced terahertz absorption, the amount of hydration water can be obtained by detecting decrease in terahertz absorption.

II. Results

Using a high-precision terahertz time-domain spectroscopy (THz-TDS) setup [1], we have studied hydration of a protein (hen egg white lysozyme; HEWL) in aqueous solutions in the presence of various ions. In fig. 1, examples of terahertz absorption spectra observed are shown. These spectra are featureless, and the magnitude of absorption coefficient depended on the species and concentration of the solutes. The absorption decrease observed in the HEWL solutions is mostly due to decrease in the amount of water in the solutions, since the proteins have terahertz absorption much less than that of water. By taking this effect into account by measuring the density of the solutions, the amount of hydration water can be estimated [2].

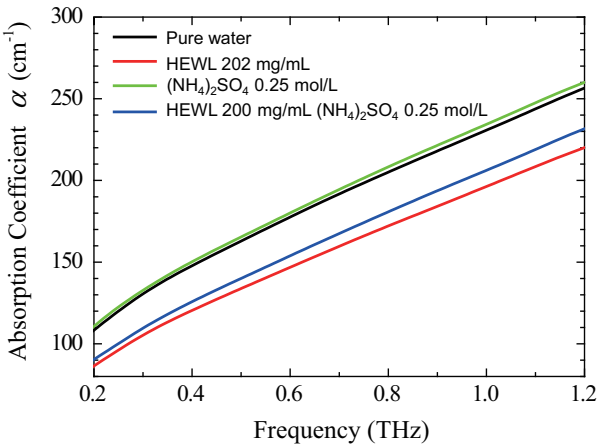

Fig. 1. Terahertz absorption spectra of pure water, HEWL aqueous solution, $(NH_4)_2SO_4$ aqueous solution, and HEWL-$(NH_4)_2SO_4$ mixed aqueous solution.

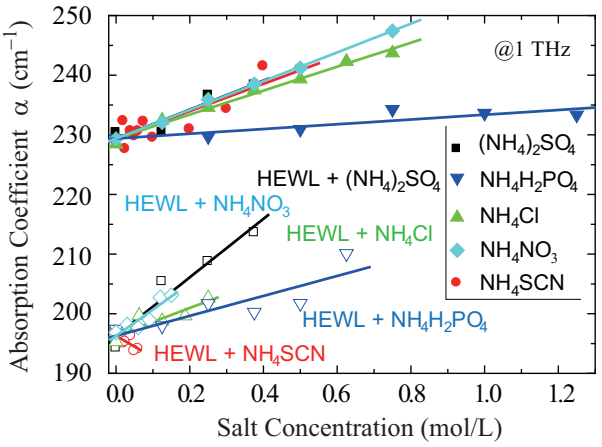

Fig. 2. Salt concentration dependence of absorption coefficient of salt aqueous solutions (upper half) and of HEWL(200 mg/mL)-salt mixed aqueous solutions (lower half) at 1 THz, shown with linear fitting lines.

The salt concentration dependence of absorption coefficient at 1 THz are summarized in fig. 2. It was confirmed, by means of Fourier-transform IR and eye observation, that no denaturation, aggregation, or precipitation of HEWL took place in the observation range of concentration of each salt.

As shown in the figure, the absorption coefficients of the salt aqueous solutions and the HEWL-salt mixed aqueous solutions increased linearly with increasing salt concentration. Difference in the slopes of the ion concentration dependence of the HEWL solutions and the HEWL-salt mixed solutions is attributed to the contribution of salt effects on the hydration water. For example, by addition of $(NH_4)_2SO_4$, terahertz absorption of HEWL solutions increases much more rapidly than that of the salt solution without protein. This shows that the terahertz absorption of water around the protein increases by the addition of this salt due to accelerated dynamics of the water. Addition of NH_4SCN, on the other hand, to the HEWL solution resulted in rapid decrease in terahertz absorption, which shows slowdown of the dynamics of water around the protein.

The slope differences between the salt solution and the HEWL-salt mixed solution depend on the anion, and are ordered consistently, with the exception of NO_3^-, with the Hofmeister series as

$$SO_4^{2-} \simeq NO_3^- > H_2PO_4^- > Cl^- > SCN^-.$$

This shows that kosmotropic anions accelerate the water dynamics around proteins, whereas chaotropic anions slow it down. These effects of Hofmeister anions on the hydration water are in contrast to their effects to bulk water, since kosmotropes strengthen hydrogen-bonding network of bulk water, and chaotropes destroy them. These behaviors are consistent with the theoretical model presented by Collins [3], where effects of anions to the hydrogen-bonding network of water is considered. It is suggestive that we observe deviation of NO_3^- from the Hofmeister series. Although NO_3^- has been categorized as a structure breaker, the current results of terahertz spectroscopy suggest that NO_3^- is a structure maker and that interaction between NO_3^- and water is strong. We can attribute the rather chaotropic positioning of NO_3^- in the Hofmeister series to its specific interaction of the nitrogen atom of this anion with those of proteins.

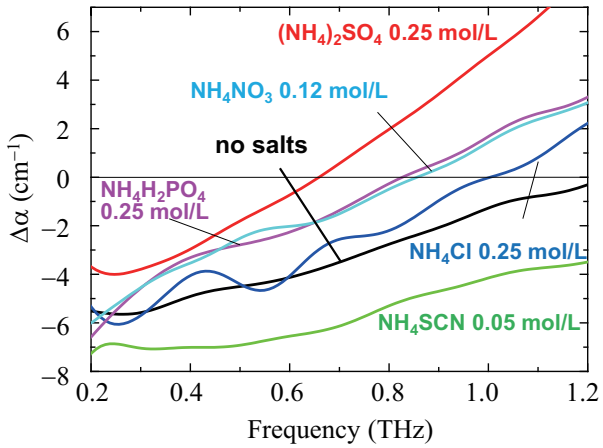

Fig. 3. Difference spectra of salt aqueous solution and HEWL(200 mg/mL)-salt mixed aqueous solutions, which are obtained by subtracting the water absorption contribution from the measured absorption coefficient.

Figure 3 shows the difference spectra $\Delta\alpha$ of salt aqueous solutions with and without HEWL. These spectra were obtained by subtracting the contribution of water (assuming bulk water absorption) from the measured spectra. The negative amount of the difference spectra in the low frequency region indicates the slower dynamics of lysozyme hydration water than that of bulk water [1], [2]. Spectra of change rate of $\Delta\alpha$ by addition of salts, A_2, were calculated from the fitting data obtained in fig. 2, at each frequency, which are shown in fig. 4. These correspond to the terahertz absorption change of water around protein due to salt addition. By achieving better data quality, terahertz spectroscopic study of hydration water would be possible.

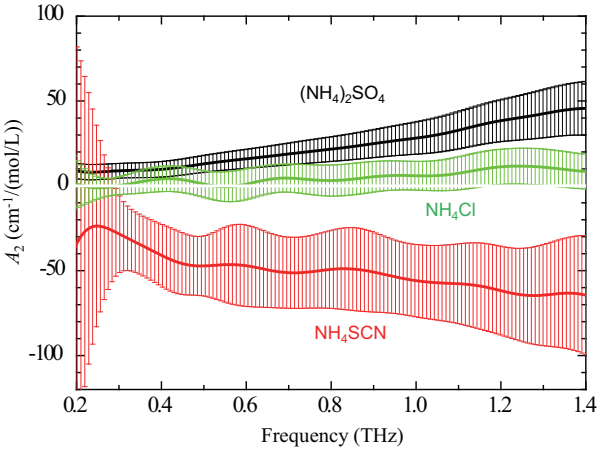

Fig. 4. Spectra of change rate of $\Delta\alpha$ by addition of $(NH_4)_2SO_4$ (black), NH_4Cl (green), and NH_4SCN (red). Vertical lines indicate error bars.

III. Conclusion

Using high-precision THz-TDS, it has been shown that kosmotropic anions increase terahertz absorption of water around protein molecules, whereas chaotropes decrease it.

The results show that kosmotropic anions make faster the dynamics of water around the protein, and that chaotropic anions make it slower. This result is consistent with the model of Collins [3], where effects of anions to the hydrogen-bonding network of water in the protein hydration shell is considered. The present results show that influence of anions on hydration is the main factor that determines the stability of proteins in aqueous solutions.

References

[1] K. Aoki, K. Shiraki, and T. Hattori, Appl. Phys. Lett. **103**, 173704 (2013).
[2] N. Q. Vinh, S. J. Allen, and K. W. Plaxco, J. Am. Chem. Soc. **133**, 8942 (2011).
[3] K. D. Collins, Methods **34**, 300 (2004).