Supporting information for "Salt effects on the picosecond dynamics of lysozyme hydration water investigated by terahertz time-domain spectroscopy and an insight into Hofmeister series for protein stability and solubility"

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Lysozyme structure characterization using Fourier transform infrared spectroscopy

Denaturation or conformation change of lysozyme may result in a change in the terahertz absorption of lysozyme. Here, we confirmed that, in our experimental conditions, lysozyme denaturation does not occur by addition of salt using Fourier transform infrared (FT-IR) spectroscopy in an attenuated total reflection (ATR) geometry. Conformational change of the protein results in a change in the spectral profile of the amide I band (1600-1700 cm^{-1}).[S1] Dong *et al.*[S1] reported deconvoluted absorption spectra of various proteins in the amide I region. Deconvolution of the spectra makes the line width of absorption peaks narrower, which enables us to find the peak positions of each peak components. They showed that α helix rich proteins have an absorption peak around 1650-1660 cm^{-1}, whereas β sheet rich proteins have an absorption peak between 1620-1640 cm^{-1}. In both

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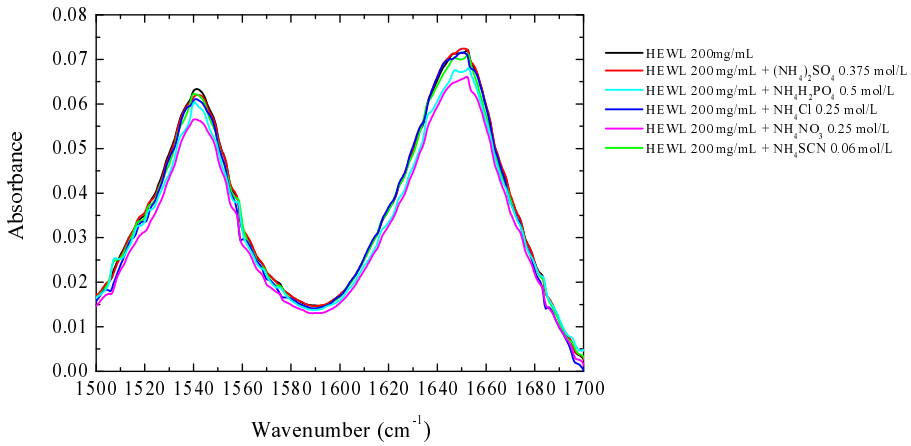

Figure S1. Absorbance spectra of lysozyme in the lysozyme-salt mixed aqueous solutions. The spectrum for NH_4SCN was measured at 20 °C and those for the other salts were measured at 25 °C.

the raw absorption spectra before deconvolution and the deconvoluted spectra, spectral profile of the absorption clearly depends on the secondary structure of the protein; α helix rich proteins have an absorption peak around 1650-1660 cm^{-1}, whereas β sheet rich proteins have an absorption peak in 1620-1640 cm^{-1}. Therefore, the peak position of the absorbance spectra before deconvolution is indicative of the secondary structure of the protein. It is expected that the spectral profile and peak position of the absorbance spectra provides information corresponding to the circular dichroism (CD) measurements. Here, we confirmed that conformation change of lysozyme does not occur from the spectral profile and peak position of lysozyme absorption in the amide I band.

Absorbance spectra of lysozyme in the lysozyme(200 mg/mL)-salt mixed aqueous solution are shown in Fig. S1. Fluctuations on the spectra are attributed to absorption by water vapor in the atmosphere. The peak in the spectra is located at 1650 cm^{-1} and is independent of salt. This result indicates that the secondary structure of lysozyme is not changed by the addition of salt.

Lysozyme concentration dependence of the absorption coefficient of the lysozyme aqueous solution

Figure S2 shows the lysozyme concentration dependence of the absorption coefficient of the lysozyme aqueous solutions. In the low concentration region, the absorption coefficient of the lysozyme aqueous solution decreased. The absorption coefficient has a peak around 50 mg/mL. A further increase in the concentration results in a monotonical decrease in the absorption coefficient. At 50 mg/mL, our analysis showed that the absorption coefficient of water around the lysozyme molecule increases. Increase of the absorption is attributed to faster dynamics of water around the lysozyme than that of bulk water.[S2] At this concentration, the distance between the centers of the lysozyme molecules is about 8 nm, when we assume that lysozyme molecules are arranged at lattice points. Since the diameter of the lysozyme molecule is about 3 nm,[S3] the distance from the lysozyme surface to the water molecules that are located at the farthest point is estimated to be about 2.5 nm. A further increase in the concentration should result in an even shorter distance. For example, the distance is about 1 nm at a lysozyme concentration of 200 mg/mL and the dynamics of water observed is expected to become slower. Therefore, it is expected that we observed changes in the dynamics of water molecules about 1 nm from the lysozyme surface in the experiments presented here.

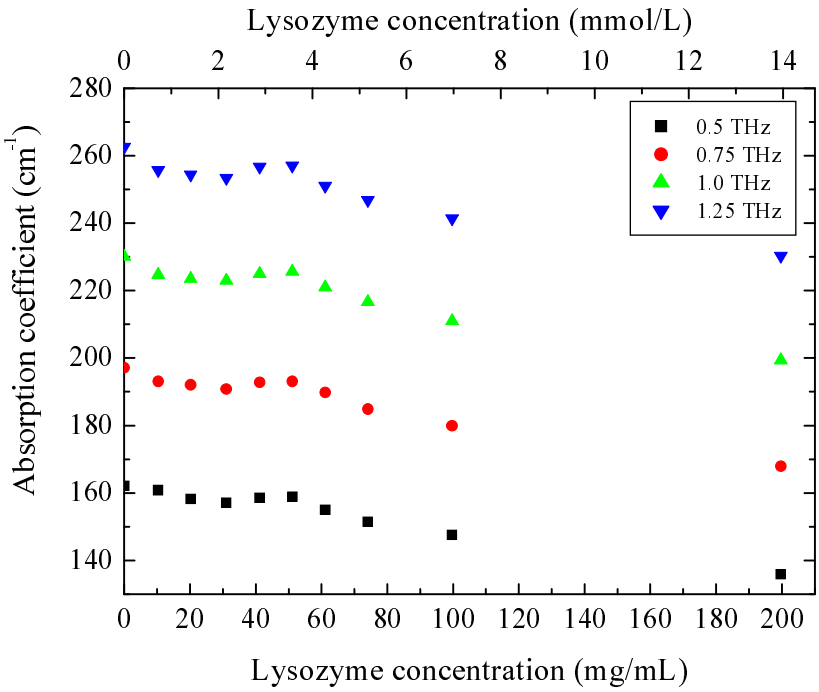

Figure S2. Lysozyme concentration dependence of absorption coefficient of lysozyme aqueous solutions.

Enlarged plots showing the absorption coefficient of lysozyme-salt mixed aqueous solutions in Figure 3

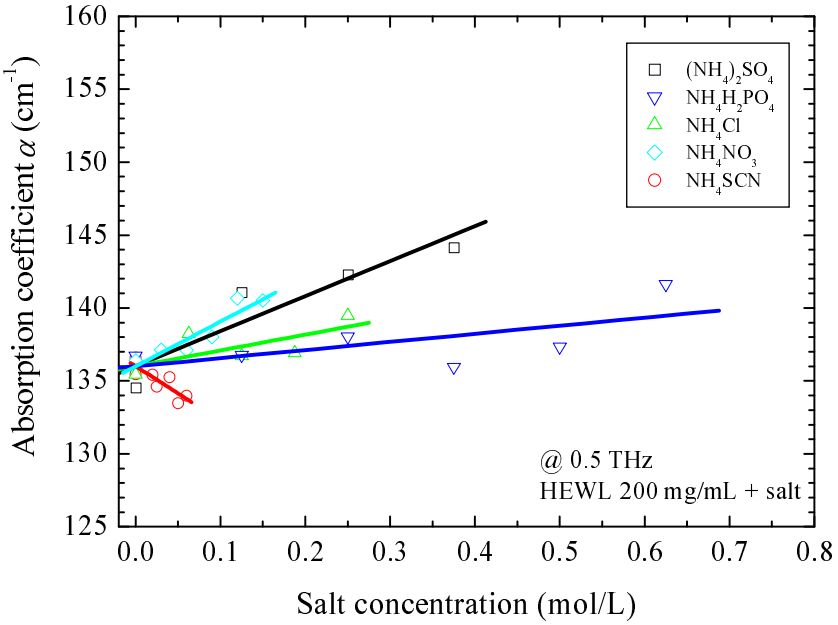

Figure S3. Salt concentration dependence of the lysozyme (200 mg/mL)-salt mixed aqueous solutions at 0.5 THz.

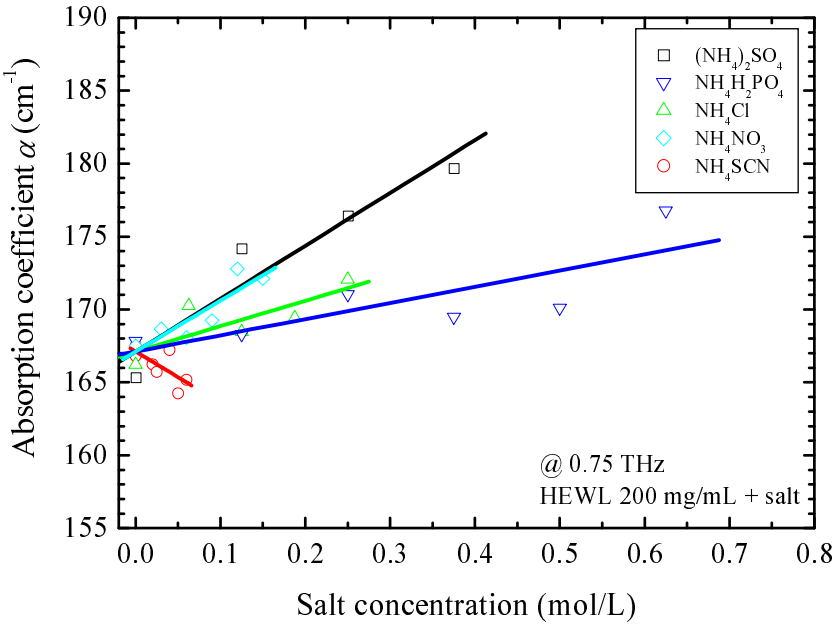

Figure S4. Salt concentration dependence of the lysozyme (200 mg/mL)-salt mixed aqueous solutions at 0.75 THz.

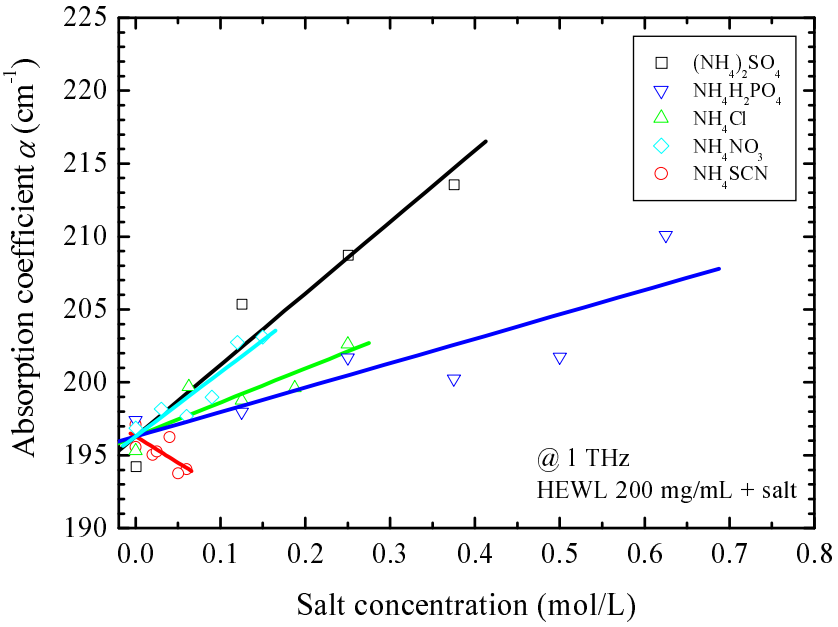

Figure S5. Salt concentration dependence of the lysozyme (200 mg/mL)-salt mixed aqueous solutions at 1.0 THz.

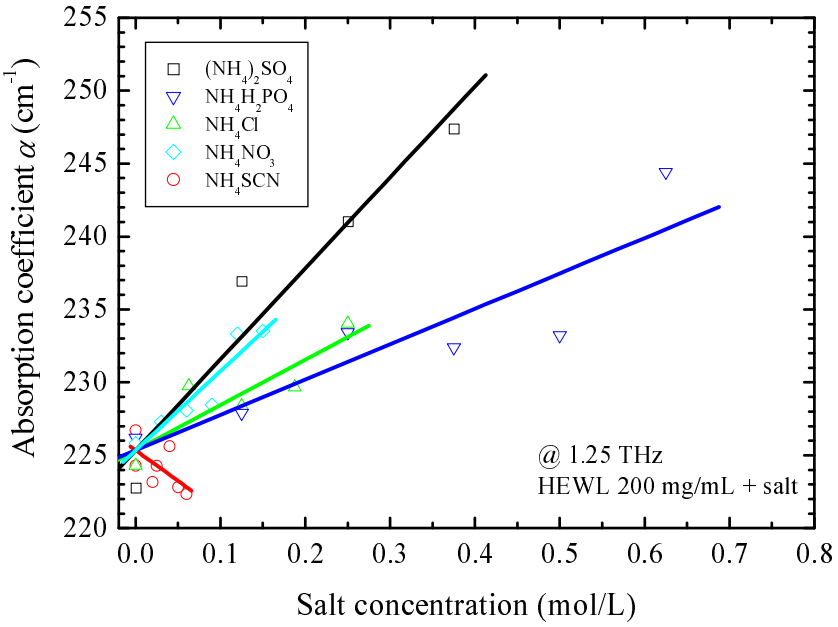

Figure S6. Salt concentration dependence of the lysozyme (200 mg/mL)-salt mixed aqueous solutions at 1.25 THz.

References

S1 A. Dong, P. Huang and W. S. Caughey, *Biochemistry*, 1990, **29**, 3303–3308.

S2 M. Heyden and M. Havenith, *Methods*, 2010, **52**, 74–83.

S3 C. C. F. Blake, D. F. Koenig, G. A. Mair, A. C. T. North, D. C. Phillips and V. R. Sarma, *Nature*, 1965, **206**, 757–761.