Observation of protein large-amplitude vibrational motions induced upon hydration

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1. Introduction

Since protein function is exhibited in the thermal fluctuation of solvent, water, it is essential to understand effects of hydration and its thermal excitation on protein dynamics to understand protein functional mechanisms. Especially, dynamics ranging from picosecond (10-12 sec) to nanosecond (10-9 sec) correspond to the energy of the thermal fluctuation, and are known to be rich in the dynamics such as rotational relaxation of hydration water and protein low-frequency vibrational modes. We have elucidated the effect of hydration and its thermal excitation on protein dynamics in the frequency regions by measuring the complex dielectric spectra obtained by two spectroscopic methods; a vector network analyzer (GHz region) and a THz time-domain spectrometer (THz region). So far we have revealed one important phenomenon; the temperature dependence in the GHz-THz frequency region is mainly governed by a rotational relaxational mode of hydration water. These observations strongly suggest that protein large-amplitude vibrational motions that might be important for protein functional expression were induced via an excitation of anharmonicity of protein low-frequency vibrational modes in the THz region by the rotational relaxational mode of hydration water in the GHz region. As a next stage, it is essential to directly observe the molecular motions related to the protein large-amplitude vibrational motions to understand the role of the dynamics for protein function. However, the technique that the applicant has been used, i.e. dielectric spectroscopy, retains one weak point for achieving the purpose; because of the large contribution of the rotational relaxational mode of hydration water to the complex dielectric spectra, a spectral component corresponding to the protein large-amplitude vibrational motions is expected to be buried, and thus it is difficult to extract the spectral component. To solve the problem, we have obtained temperature-dependent neutron-scattering spectra of lysozyme hydrated by deuterated water, using BL02, DNA.

2. Experiment

Commercially-available hen-egg white lysozyme was dialyzed by distilled water and lyophilized. The lyophilized power was dissolved in D_2O and lyophilized, which was repeated three times. The solvent-deuterated power was then hydrated by vapor D_2O, resulting in obtaining two hydrated powers where the value of h = 0.39 or 0.52, respectively. The value of h corresponds to a value of mass of water divided by mass of protein. The two hydrated samples as well as a dehydrated sample (h = 0.11) were used for the neutron scattering experiment. The samples were sandwiched by aluminum foil and placed in an aluminum cylinder cell The neutron-scattering experiment was performed using BL02, DNA equipped with a cryostat with turning of its high-speed chopper. The energy or q range was -0.5 < E < 1.5 meV or 0.1 < q < 2.0 Å, respectively. The measurements were firstly performed by increasing temperature up to 293 K and then decreasing. Experimental temperature points were as follows;

h = 0.11; (T-up) 233, 243, 253, 263, 273, 283, 293, (T-down) 283, 273, 263, 253, 243, 233, 10 (K)

h = 0.39 and 0.52; (T-up) 233, 243, 253, 263, 273, 283, 293, (T-down) 283, 273, 263, 253, 243, 233, 203, 173, 143, 113, 83 (K)

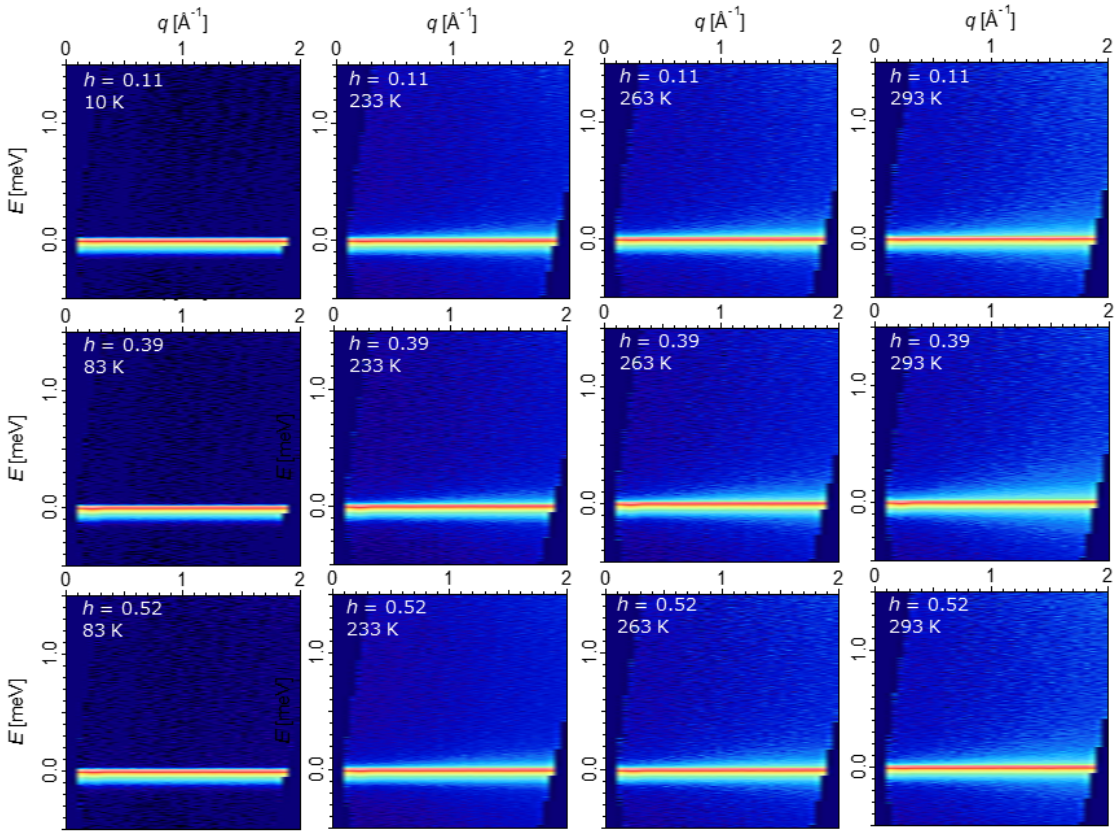
For the analysis of the spectra, a scattering spectrum of a vacant cell (aluminum foil only) at 293 K was also obtained and subtracted from the sample data.

3. Results

The figure below shows *E*-*q* spectra of dehydrated and hydrated lysozyme. In the dehydrated state, it can be seen that quasielastic scattering component slightly increases as a function of temperature. Surprisingly, the hydrate sample also showed similar spectra at each temperature point. This is inconsistent with a couple of previous results where quasielastic components dramatically increased above ~200 K compared to a dehydrated state observed in various kinds of proteins (Schirò *et al*., *Nat. Commum*. 6:6490 (2015)). To quantify the degree of the contribution of the quasielastic component, we fit representative *E*-dependent spectra where the spectral components along the q value was integrated at q = 1.0±0.1. The model function used for the fitting was composed of an elastic component, a quasielastic component, and background shown as follows;

$$S(E)\,|_{Q=1.0\pm0.1} = \left[A_0 \cdot \delta(E_0) + \frac{A_{\mathrm{lor}}}{\pi} \cdot \frac{\Gamma_{\mathrm{lor}}/2}{(E_0 - E)^2 + \Gamma_{\mathrm{lor}}/2} \right] \otimes R(E) + BG$$

where $\delta(E_0)$ and A_0 represent the delta function and its amplitude, representing the elastic-scattering component, in which the scattering becomes the largest at E_0. A_{lor} and Γ_{lor} represent the amplitude and the full-width-at-half-maximum (FWHM) of the Lorentzian function, which corresponds to the inelastic-scattering component. BG implies the background constant. It turned out that FWHM was 4.3±0.1×10^{-2} meV, 4.7±0.1×10^{-2} meV, or 4.3±0.1×10^{-2} meV for h = 0.11, h = 0.39, or h = 0.52, respectively. This result clearly shows that the quasi-elastic component did not apparently increase upon hydration. One probable possibility is that hydration water reacted with aluminum foil, which changed to alumite.



4. Conclusion

To prevent the reaction between hydration water and aluminum foil, different materials such as vanadium has to be used to wrap and possibly enclose the hydrated powers. Now we are discussing about how to modify the sample cell.