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# The methylene reorientation of Lysozyme at the Air-Solution Interface Studied by Broadband Sum Frequency Generation Vibrational Spectroscopy

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## Introduction

It has been proven that the terminology of "hard" and "soft" is useful to describe the tendency of a certain protein to undergo major surface-induced conformational change. As a hard protein, lysozyme (LSZ) has four internal disulfide bonds to help the maintenance of its tertiary. [1] Neutron reflectivity study has shown that the adsorbed LSZ molecules at air-water interface retain their globular framework.[2] FTIR spectroscopy study has found that, however, the formation of an unfolded protein network happens within 10min, where it contains many antiparalled $\beta$-sheet structure.[3] Although many works have been performed on this topic, there is still in lack of the information of interfacial side-chain reorientation of LSZ. These informations will shed insight on the LSZ conformational change and the unfolding process at the interfaces. Recently, sum frequency generation (SFG) spectroscopy has been used to study protein adsorption at air-water interface and solid water interface.[4] Broadband SFG (BBSFG) spectroscopic systems can offer shorter acquisition times and larger SFG responses than many scanning SFG system.

## Experiments section

In our BBSFG setup, we used a broadband IR beam ($\Delta\sim$250cm$^{-1}$, 250fs, 10$\mu$J/puls) and a narrow band visible beam (800nm, 10ps, 1mJ/pulse) introduced on the sample surface. The incident angles of the IR beam (P-polarized) and the visible one (S-polarized) were 49° and 60°, respectively. The S-polarized component of the BBSFG signal was detected with a thermoelectric cooled CCD. The acquisition time of each spectrum was 2min.

We purified the LSZ-PBS solution of 30mg mL$^{-1}$ and diluted them to 1mg mL$^{-1}$ and 0.01mg mL$^{-1}$. The diluted solution was shook strongly in a glassware for homogeneity. The LSZ-PBS solution was poured into a dish and soon afterward SFG detection was started. The time point of pouring was recorded as time-zero.

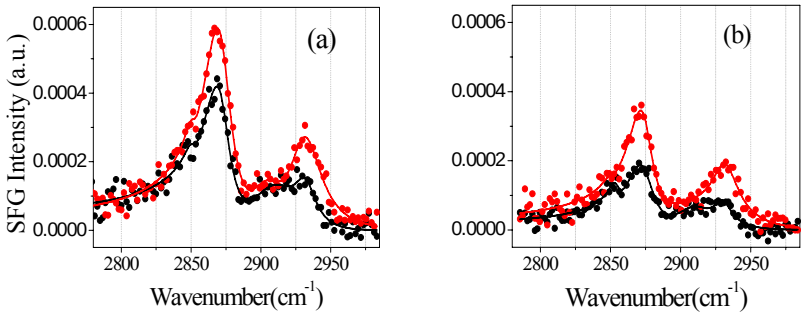



Fig.1 SFG spectra collected from the air-LSZ solution interfaces with different LSZ concentrations and different aging time (ssp). Solide lines are the fitting results. (a) 1mg mL$^{-1}$ black: 8min. red 600min. (b) 0.01mg mL$^{-1}$ black: 50min. red 600min.

## Results and Discussion

Shown in Figure 1, are the SFG spectra from the air-solution interface of two different concentrations of LSZ ((a) 1mg mL$^{-1}$ and (b) 0.01mg mL$^{-1}$). Four features can be identified from the fitting results. The main peak at 2872 cm$^{-1}$ and the weaker peak at 2933cm$^{-1}$ were assigned to the symmetric stretch (ss) and Fermi (F) resonance stretch vibrations of the terminal $CH_3$ group of the side chains, respectively. The

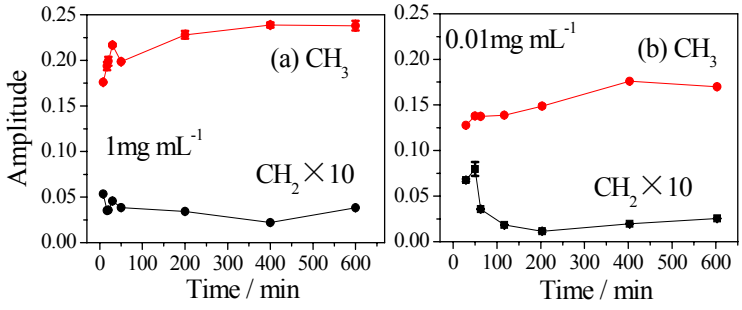



Figure 2. The kinetics of the SFG amplitude of $CH_3$ (red) and $CH_2$ (black) ss modes at the bulk concentrations of 1mg mL$^{-1}$ (left) and 0.01mg mL$^{-1}$(right).

shoulders around 2850 and 2914cm$^{-1}$ were assigned to the symmetric stretch (ss) and antisymmetric stretch (as) vibrations of $CH_2$ group respectively.

Moreover, we compared the time-dependence of the amplitude of $CH_3$(ss) and $CH_2$(ss) at the two different concentrations, as shown in Fig.2. For the case of 1mg mL$^{-1}$, the first time point is 8min, but it is 29min for the case of 0.01mg mL$^{-1}$, because the spectra before 29min are too noisy to be fitted.

Shown in Fig.2a is the amplitude variation of the 1mg mL$^{-1}$ LSZ. The $CH_3$(ss) mode increases with adsorption time, while $CH_2$(ss) has a little bit trend of decrease. In Fig. 2b, the $CH_3$(ss) mode in the 0.01mg mL$^{-1}$ LSZ case increases with the adsorption time in a similar manner. But a drastic decrease of the amplitude of $CH_2$(ss) has been observed at the initial stage of adsorption.

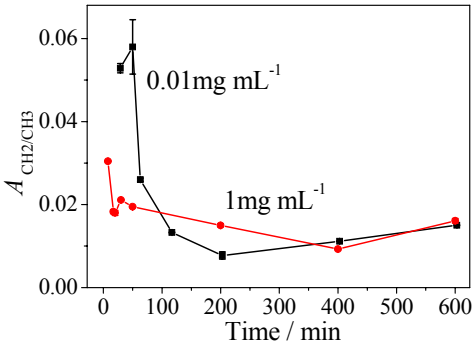



Figure 3. The time dependence of the ratios of the oscillator strengths of the $CH_2$ (ss) and $CH_3$ (ss) modes at the bulk concentrations of 1mg mL$^{-1}$ (●) and 0.01mg mL$^{-1}$(■).

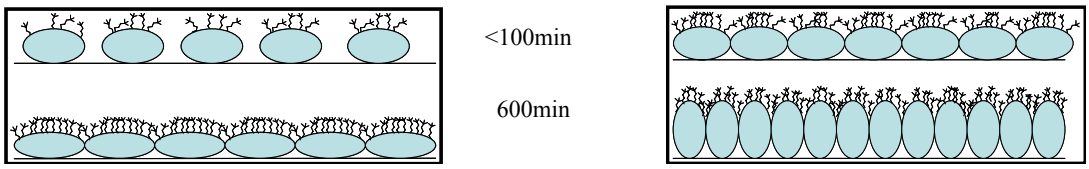
For more accurate analysis, we calculated the evolution of oscillator strengths ratio, $A_{CH2(ss)}/A_{CH3(ss)}$, which corresponds to the evaluation of the contribution of $CH_2$ mode, as shown in Fig. 3. From this figure, the evolutions of the contribution of $CH_2$(ss) are different for different LSZ bulk concentrations. For 1mg mL$^{-1}$, there is a small and fast decrease at 8-20min, then it almost stays constant. For 0.01mg mL$^{-1}$, the ratio decreases drastically until 200min and does not change too much later. We thought the decrease at the early stage implies a surface-induced protein conformational change. At a high concentration case, the initial change has almost finished before 20min.

In an "all-trans" configuration, the $CH_2$ vibration mode is inactive in SFG. Therefore, this $CH_2$ decrease is caused by the transformation from gauche to all-trans configuration. At the air-water interface, the hydrophobic interaction between the air and the hydrophobic side-chains of proteins, as well as protein-protein interaction makes the LSZ molecules gradually rearrange themselves and expose more $CH_3$ groups into the air. Therefore the surface becomes more crowded with the LSZs. The $CH_2$ chains have no more room to keep the gauche defect and rearrange themselves to all-trans configuration.

Table.1

| | 1mg mL$^{-1}$ | 0.01mg mL$^{-1}$ |
|---|---|---|
| surface coverage | 0.248$\mu$g cm$^{-2}$ | 0.12$\mu$g cm$^{-2}$ |
| *N* | 9-18 | 11-22 |

Following Wang's method,[4] we compared the intensity of $CH_3$(ss) of LSZ with hexadecanol monolayer on water surface. One LSZ molecule has 61 methyl groups. By using the surface coverages of the neutron reflectivity study,[2] we obtained the number of the $CH_3$ groups on one LSZ molecule which contribute to SFG signals (*N*), as shown in Table 1. From these results in Table 1, we propose a model for the orientation of methyl and methylene groups in LSZ of 0.01mg mL$^{-1}$ and 1mg mL$^{-1}$. For a native LSZ, it is impossible




0.01mg mL$^{-1}$ 1mg mL$^{-1}$

Scheme 1. Proposed model for the orientation of methyl and methylene groups in LSZ of 0.01mg mL$^{-1}$ and 1mg mL$^{-1}$.

to make 11-22 $CH_3$ pointing into the air. Therefore, we concluded that at the mesoequilibrium state the LSZ molecules have lost or partially lost their native structure for the case of 0.01mg mL$^{-1}$.

## References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