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New Insight into the Surface Denaturation of Protein: An Electronic Sum Frequency Generation Study

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Introduction:

When a protein molecule reaches the air-water interface, there is a strong tendency for the hydrophobic parts to go to the air side, which leads to unfolding of the protein. This phenomenon is called surface denaturation and has been well known in natural science from very early days.[1] Although the characterization of structure and conformation of many protein molecules have been done in the bulk, the *in situ* study of protein conformation at the interfaces have not been done due to lack of interface specific technique. Second-order nonlinear spectroscopy is a powerful tool to study the structure of molecules at interfaces.[2] We have recently developed the nonlinear multiplex Electronic Sum Frequency Generation (ESFG) technique (see figure 1) to measure interface specific electronic spectra.[2]

In this work, we have applied ESFG to *in situ* detection of protein conformation at air-water and silica-water interfaces for the first time, using cytochrome c as a model protein.

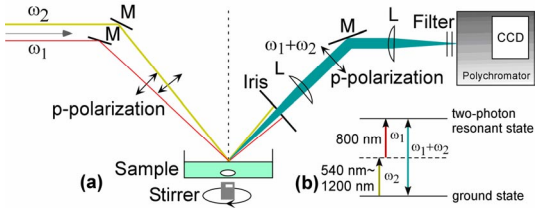

Fig. 1. (a) Ray diagram of the multiplex ESFG setup. ω_1 is 800 nm (bandwidth: 160 cm^{-1}) and ω_2 is white light continuum (540 nm ~ 1200 nm). $(\omega_1+\omega_2)$ is the ESFG signal. (b) Energy diagram of ESFG process.

Results and Discussions:

Horse heart cytochrome c is a well-characterized globular protein both in the crystalline state and in solution state.[3] The heme absorption band of the protein reflects its conformational state. The heme absorption band is centered at 410 nm and 394 nm for native and denatured protein, respectively.

Figure 2a shows the ESFG spectrum of native cytochrome c (pH=7) at the air-water interface. It is readily seen that the surface

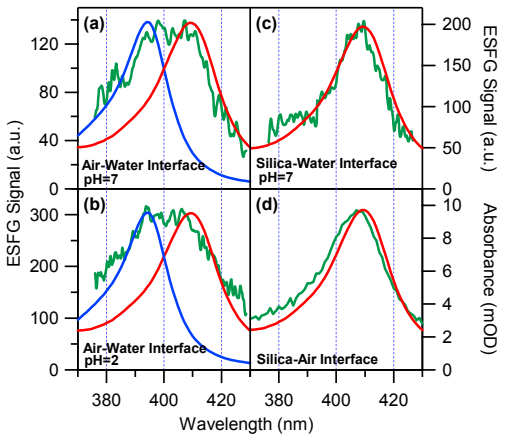

Fig. 2. ESFG spectra of cytochrome c at (a) air-water interface, bulk pH=7, (b) air-water interface, bulk pH=2, (c) silica-water interface, bulk pH=7. Green lines represent ESFG spectra. Red and blue lines represent the UV-vis absorption spectra of native and denatured protein, respectively. Absorption spectrum of a sub-monolayer film of cytochrome c at a silica-air interface (d) is also shown.

spectrum is quite different from the native-state spectrum of the protein in bulk water. The conformation of the protein at the air-water interface is certainly different from that in the bulk environment. It should be noted that the surface Soret spectrum is also very different from the Soret spectrum of the denatured protein in the bulk. The surface spectrum is very broad and the maximum position is in between the native and denatured-state absorption maxima. The broad Soret spectrum may be explained in terms of the presence of mixed conformation at the air-water interface. Figure 2b shows the ESFG spectra of cytochrome c at the air-water interface measured under an acidic condition (pH = 2). Surprisingly, the spectrum is very similar to the spectrum measured under the neutral condition, although the protein is denatured in the bulk.

We have also measured the ESFG spectrum of cytochrome c at silica-water interface (Fig. 2c). It is readily seen that at this interface the spectrum is very similar to the native-state spectrum of the protein, indicating the retention of the native structure at the silica-water interface. To check the conformation of cytochrome c at the silica-air interface, we have measured the Soret absorption spectrum of sub-monolayer film on silica surface (Fig. 2d). Although the interface is formed by two extreme polarity substrates, the observed spectrum is very similar to the native state spectrum of the protein. The roughness of the silica surface may be responsible for the stability of the protein at silica-air interface.

In summary, we have measured the ESFG spectra of a protein, cytochrome c, at the air-water and silica-water interfaces and correlate them to the conformation of the protein at the interfaces for the first time. The existence of multiple conformation of cytochrome c at the air-water interface was found under both neutral and acidic conditions. In contrast, at the silica-water and silica-air interfaces, no denaturation was detected.

References

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