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Investigating the Adsorption Behavior of Hematin at Lipid/Water Interface Using Heterodyne-Detected Electronic Sum Frequency Generation Spectroscopy

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[Introduction]

The formation of malarial pigment (hemozoin) is an important detoxification mechanism for blood feeding parasites. The hemozoin structure consists of a crystalline centrosymmetric μ- propionato dimer of hematin, and it has been proposed that dimerization is induced by the lipid membrane interface of digestive food vacuoles present within the parasite body.[1] However, such interfacial regions are often only a few molecular diameters in thickness and hence difficult to investigate experimentally. Heterodyne-detected Electronic Sum Frequency Generation (HD-ESFG) is a novel nonlinear spectroscopic tool which allows us to selectively monitor such interfacial phenomena.[2]

In this presentation, we report our attempt using HD-ESFG to monitor the hematin adsorption at lipid/water interface to examine the proposed mechanism of hemozoin formation.

[Experiment]

Figure 1A shows the chemical structure of hematin used in our studies. All measurements were carried out at a pH ~ 5.6. This is close to the pH of digestive food vacuoles in malarial parasites where hemozoin crystal formation takes place via dimerization of hematin.

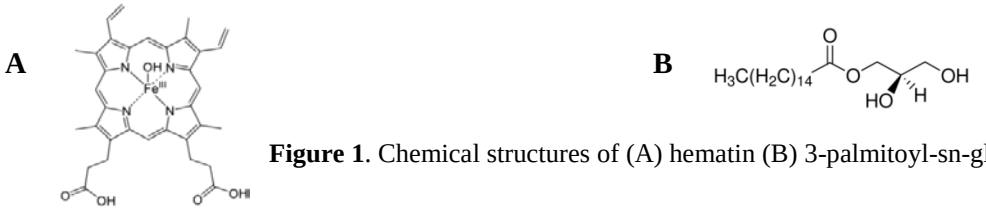

Figure 1. Chemical structures of (A) hematin (B) 3-palmitoyl-sn-glycerol

For lipid, we chose a single chain nonionic lipid, namely, 3-palmitoyl-sn-glycerol (henceforth abbreviated as PG, Figure 1B). Such monoacyl lipids have been reported to facilitate dimerization of hematin.[1] For our HD-ESFG experiments, the sample was excited simultaneously with a pair of pulses: i) a broadband visible pulse with frequency ω_2 (540 nm ~ 1.2 μm) and ii) a narrowband visible pulse with frequency ω_1 (795 nm). The sum frequency ($\omega_1 + \omega_2$) generated from the sample was subsequently mixed with sum frequency light from a local oscillator to achieve heterodyne detection. The sum frequency ($\omega_1 + \omega_2$), ω_1, ω_2 were s-, p-,

and s- polarized respectively. In this technique, when spectra are measured under two photon ($\omega_1 + \omega_2$) resonant and one photon (ω_1 or ω_2) nonresonant conditions, the Im [$\chi^{(2)}$] spectra plotted against $\omega_1 + \omega_2$ represent interfacial electronic spectra that can be directly compared with absorption spectra ([$\chi^{(1)}$]) in bulk solution.

[Results and Discussion]

Figure 2A shows the Im [$\chi^{(2)}$] spectra of 20 μM hematin solution (pH 5.6) at bare air/water interface. The peak near 410 nm is characteristic of porphyrin-based compounds and can be attributed to the Soret band. This band intensity decreases spontaneously on the time scale of several minutes after a fresh hematin solution is prepared. Independent measurement of surface tension for hematin solution indicates that hematin remains adsorbed at air/water interface for more than half an hour. Since ESFG intensity decreases but adsorbed hematin remains at interface, it is possible that this change arises from the formation of hematin dimers which do not have $\chi^{(2)}$ activity due to their centrosymmetrical structure. However, randomization of the hematin's orientation at the interface can also induce the intensity decrease, and this possibility cannot be ruled out.

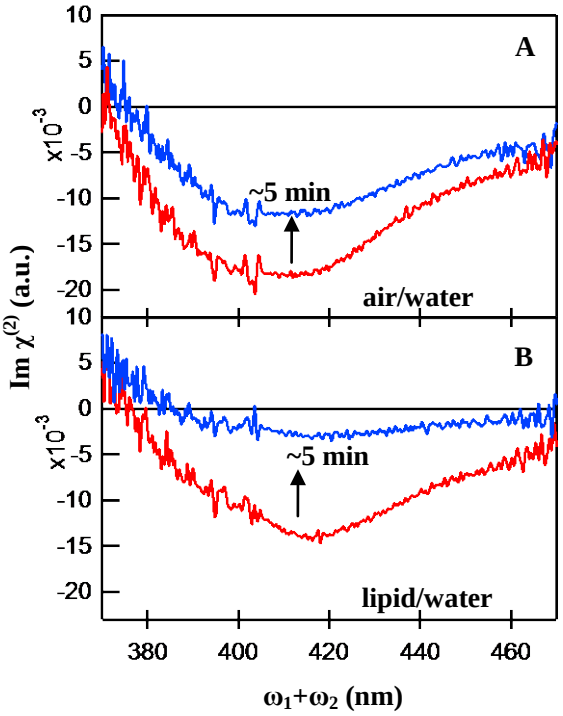

Figure 2. Im $\chi^{(2)}$ spectra of hematin at air/water interface (A) without and (B) with PG lipid monolayer (red); the corresponding spectra after 5 minutes (blue). The pH of the solution is 5.6.

In presence of a nonionic palmitoyl glycerol (PG) lipid, the decrease in hematin intensity is more substantial within the same time interval (Figure 2B). Thus, presence of a lipid monolayer at the interface is found to accelerate the decrease in ESFG intensity. Heterodyne-detected Vibrational Sum Frequency Generation (HD-VSFG) measurements in the CH and CO stretch regions indicate that alignment of the hydrocarbon tails in the lipid monolayer gets perturbed in presence of hematin. This observation suggests the presence of adsorbed hematin at the lipid/water interface. Thus, in absence of desorption, the observed acceleration of ESFG intensity decrease may indicate the acceleration of hematin dimer formation with the aid of lipid. Further experiments are underway to clarify this point.

[References]

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