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In situ Observation of the State and Stability of Hemoglobin Adsorbed onto Glass Surface by Slab Optical Waveguide (SOWG) Spectroscopy

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***Abstract*—**The state and stability of hemoglobin adsorbed on the glass surface was investigated using slab optical waveguide (SOWG) spectroscopy. The peak position of the absorption band of hemoglobin adsorbed on the glass surface was same as that of the hemoglobin in solution. This result suggests that no significant denaturation occurred by adsorption. The adsorption of hemoglobin is relatively strong that the hemoglobin molecules even remained adsorbed after rinsing the cell with buffer solution. The peak shift caused by the reduction of adsorbed hemoglobin was also observed.

***Keywords*—** hemoglobin, reduction, slab optical waveguide spectroscopy, solid/liquid interface.

I. Introduction

In situ information about surface immobilized proteins is very important in the progress of biological applications such as affinity chromatography, protein chips and biosensor, etc. Several interactions of biomolecules were investigated by using these methods in bioscience and medical field. To make preparations of the methods listed above, it is essential to adsorb or immobilize the biomolecules such as proteins on the sensor surface or support materials. The conditions for adsorption or immobilization of proteins were suffered from the difference of solution components and the surfaces. The state of the immobilized proteins were known to closely related with the degrees of the affinity between solid surface and proteins. An unexpected denaturation of adsorbed proteins would be occurred occasionally depend on the environment of the support matrices. It is therefore necessary to obtain in situ information on surface immobilized proteins to confirm their structures and properties at interfaces. UV/Vis spectroscopy is one of the essential methods for structural and functional investigation of the entire molecules. However, it is difficult to observe the absorption spectra for thin layers of proteins adsorbed on the surfaces because of the lack of sensitivity of conventional methods.

Slab optical waveguide (SOWG) spectrophotometer has been developed as one of the most important devices to investigate the interfacial phenomena [1]-[4]. The mechanisms and properties of SOWG were as follows.

The evanescent wave generated on the surface of the SOWG when light is transmitted through its core selectively interacts with the material near the surface. Also, since multiple reflection is used in optical propagation in the core of the SOWG, its sensitivity is extremely high. The SOWG spectroscopy developed by the authors [2], [5]-[11] can propagate white light with a wide wavelength range from the ultraviolet to the visible region (350-700 nm) and is suitable for in situ measurement of the absorption spectrum of very small amounts of materials adsorbed on the surfaces. Moreover, it is possible to attribute and distinguish the adsorbates according to its specific absorption spectra.

Our research group has demonstrated that it is easy to obtain in situ absorption spectra of materials adsorbed on solid/liquid interfaces and to investigate interfacial phenomena by SOWG [2], [5]-[11]. For example, analysis for adsorption and desorption process of hemoglobin [8]-[10] and electrochemical states of adsorbed cytchrome *c* have been investigated by our group [11] and others [12], [13].

Hemoglobin is a tetrameric globular protein which containing heme moiety with a molecular weight of 64,500 and isoelectric point at 6.8. Hemoglobin is chosen as a model protein for observing the state and stability of the adsorbed protein on the solid surface because it has strong spectroscopic signatures. Additionally, hemoglobin is easily to adsorb on a hydrophilic surface, which allows a bare-glass SOWG to be used directly without any chemical modification [9].

In this study, the state and stability of hemoglobin adsorbed on the bare glass surface was investigated for in situ absorption spectra obtained using SOWG spectroscopy.

II. Materials and Methods

A. Materials

Hemoglobin (bovine) was obtained from Sigma Chemical Co. (St. Louis, MO). Hemoglobin solution (10 μM) was prepared in 10 mM phosphate buffer (pH 7.4). When not in use, the hemoglobin solution was stored in a refrigerator at 4 ˚C.

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Sodium dithionite ($Na_2S_2O_4$) and sodium disulfite ($Na_2S_2O_5$) were from Nacalai tesque and KANTO CHEMIKAL Co., INC., respectively. Both reagents were dissolved in the phosphate buffer to give a concentration of 10 mM.

B. Optical Spectroscopy and Measurements

SOWG spectroscopic experiments were performed using the system fabricated in our laboratory described previously. A 50 μm thick glass plate (Matsunami Glass Industry, Ltd., Japan) was used as SOWG. A Xe 150 W Lamp Unit (SIC, Japan) was used as the light source, while the detector was a CCD from Hamamatsu Photonics (Model C7473, Japan). The incident light is conveniently guided into a SOWG by immersing the tip of an optical fiber from the light source in a droplet of glycerol laid on top of the glass surface. The schematic diagram of the SOWG spectrometer used in this study is depicted in Fig. 1. Before each experiment, glass SOWG were rinsed gently with pure water (Milli-Q) and then with ethanol. The sample was introduced through a 10 × 5 mm rectangular hole made of silicone rubber sheet mounted on top of the glass surface. The glass surface to be used was equilibrated with the same buffer solution as the protein solution to be studied until the blank absorbance stayed at a zero value. The sample was gently introduced into the cell. Absorbance was calculated from the equation $A = \log(I_R/I_S)$, where I_R and I_S are the reference and the sample intensities, respectively, at a wavelength. The method for monitoring the reduction of adsorbed hemoglobin is as follows. After the equilibrium of the SOWG surface by 10 mM phosphate buffer, the 10 μM hemoglobin solution (100 μl) was first introduced into the SOWG cell. Then, the absorption spectrum was measured for 2 min. Subsequently, all hemoglobin solution was removed from the cell, without being allowed to dry, the cell was refilled with 10 mM sodium dithionite solution (100 μl) and the SOWG absorption spectrum was again measured. The absorption spectra was collected every 1 s time interval after sample introduction. All experiments were performed at room temperature.

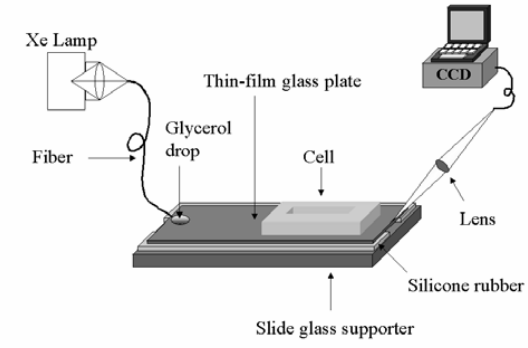

Fig. 1 Schematic diagram of the slab optical waveguide (SOWG) spectrometer.

III. Results and Discussion

A. Adsorption of hemoglobin onto the bare glass surface

When 10 μM hemoglobin solution was applied on the SOWG cell, the absorbance peak is clearly observed (Fig.2 a), which means hemoglobin molecules were adsorbed on the glass surface. In contrast, when 10 mM phosphate buffer was introduced in the SOWG cell as sample, there were no significant changes in the absorption spectra during the measurement time (Fig.2 b).

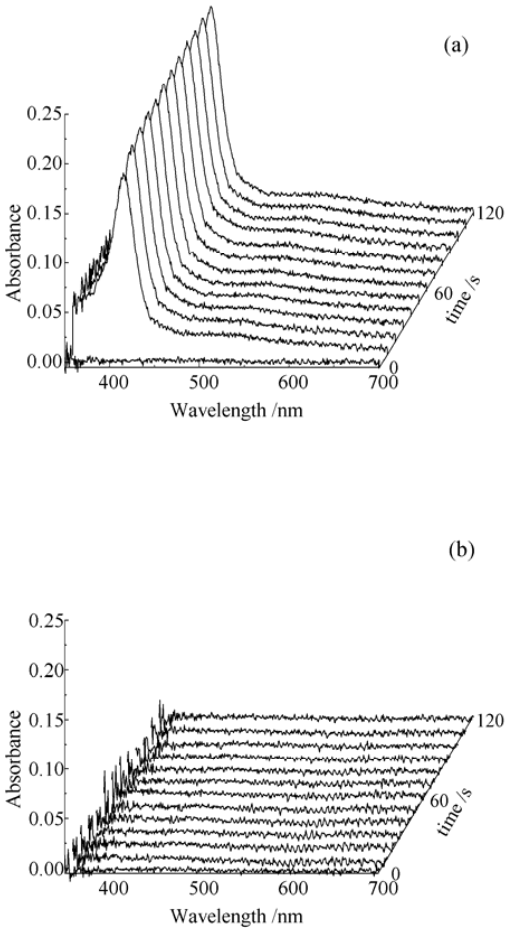

Fig. 2 Absorption spectra of adsorbed hemoglobin from 10 μM solution (a) and 10 mM phosphate buffer (b) observed by slab optical waveguide (SOWG) spectroscopy. Superimposed absorption spectra collected at 10 s time intervals for 2 min.

The absorbance peak of adsorbed hemoglobin appears around at 406 nm. This result agrees with the value previously reported [14]-[17]. The absorbance spectrum in the Soret

region results mainly from the interaction between the heme moiety and well-defined tertiary structure and hence has been used to monitor protein unfolding [18]-[21]. The conformational change of hemoglobin adsorbed on the solid surface or conjugated with the inorganic support materials such as silica or clays were reported in the previous works [22]-[24]. For example, Kondo et al. observed the conformational change of hemoglobin adsorbed on the silica, titania and zirconia ultrafine particles after 2.5 h incubation by using the circular dichroism (CD) spectra and absorbance spectra [22], [23]. On the other hand, the structure of hemoglobin encapsulated in silica gels and powders were estimated from the Uv/vis absorption spectra by McCool et al [24]. As shown in the previous works, when hemoglobin was denatured by adsorption, the absorbance spectrum of the Soret band was shifted or disappeared. In our case, the SOWG spectrum of adsorbed hemoglobin was the same as that of native hemoglobin in solution [14]-[17]. This result suggests that no significant denaturation occurred by adsorption.

B. Effect of the rinsing with buffer to adsorbed hemoglobin molecules

In order to study the strength of the adsorption of hemoglobin on the glass surface, the sample solution was removed from the SOWG cell after hemoglobin adsorption, and the buffer solution was re-introduced into the SOWG cell. Fig. 3. shows the absorbance at 406 nm versus the measurement time.

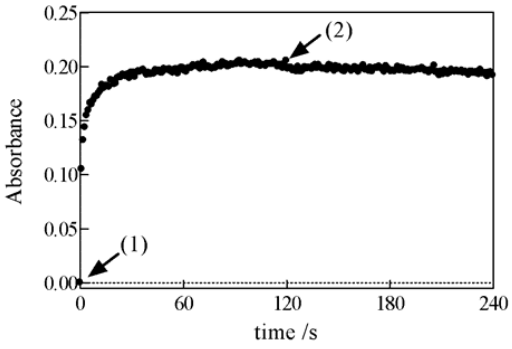

Fig. 3 Time dependent absorbance change of hemoglobin observed by SOWG spectrophotometer. (1) Introduction of hemoglobin solution; (2) subsequent removal of protein solution and addition of buffer solution.

The absorbance value increase with time, indicating increased numbers of adsorbed hemoglobin molecules. Subsequently, all hemoglobin solution was removed from the SOWG cell and the buffer solution was re-introduced into the cell. When the solution was exchanged, the absorbance at 406 nm was observed to slowly decrease with time, indicating a slight desorption of hemoglobin molecules from glass into buffer solution. As described above, the decrease of the absorbance intensity was slightly, it was thought that the almost of adsorbed hemoglobin molecules sill remain on the glass surface. The adsorption of hemoglobin on the glass surface was relatively strong that the molecules even remained adsorbed after rinsing the cell with buffer solution.

C. Observation of the state of adsorbed hemoglobin

For further understanding of the state of adsorbed hemoglobin, the spectral changes of adsorbed hemoglobin caused by exchanging sample buffers were obserbed. It is known that hemoglobin is reduced by sodium dithionate [8], [24], [25]. The reduction of adsorbed hemoglobin on octyltrichlorosilane (OTS) functionalized quartz SOWG was reported previously by Yoshida et al. [8]. But there was no data and the reduction behavior was outlined by only explanation. As shown in Fig. 4, the first 2 min as the adsorption process of hemoglobin, the peak wavelength is observed at around 406 nm. When the solution was replaced to the 10 mM sodium dithionite, the peak wavelength was dramatically shifted to longer wavelength around at 430 nm. This value is consistent with the published data for reduced hemoglobin [8], [26], [27]. Moreover, this absorbance value is stable during the measurement time. It was thought that the adsorbed hemoglobin on the glass surface was reduced by sodium dithionite solution. As a control experiment, the substitution of the solutions were performed by 10 mM sodium disulfite solution and 10 mM phosphate buffer. But in both case, no significant peak shift were observed (data not shown). In these conditions, the reduction of adsorbed hemoglobin did not occurred, and it was considered that the adsorbed hemoglobin molecules maintained its initial phase. As shown here, in situ information about the difference of the state of adsorbed protein was observed by SOWG spectroscopy.

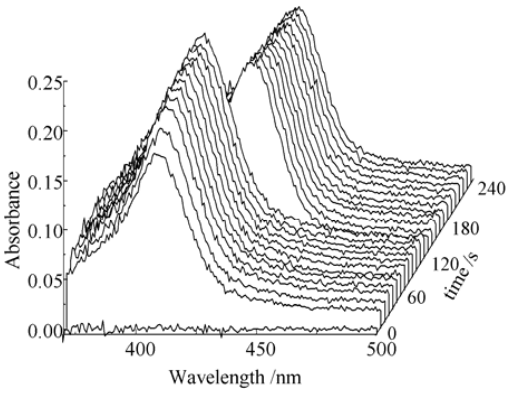

Fig. 4 Spectral change at Soret region of the adsorbed hemoglobin observed by SOWG spectroscopy. Superimposed absorption spectra collected at 10 s time intervals for 4 min.

IV. Conclusion

In situ absorption spectra for hemoglobin adsorbed on the bare glass surface were successfully obtained using SOWG spectroscopy. It was found that hemoglobin molecules adsorb strongly on the glass surface and maintained its native form in this experimental condition. Further study about the states of adsorbed hemoglobin on the various functionalized SOWG surfaces is now in progress.

The state of the adsorbed proteins is directly connected with the performance of the several important and novel applications in bioscience. This method allowed in situ investigation of the states and stability of adsorbed proteins on the surfaces. As a result, the conditions of the adsorbed proteins were clearly revealed. Concerning to the application of SOWG spectroscopy method for the bioassays, the reliability must be highly improved.

References

[1] K. Ito, A. Fujishima, "An application of optical waveguides to electrochemistry," in *J. Phys. Chem.* Vol. 92, 1988, pp. 7043-7045.

[2] K. Kato, A. Takatsu, N. Matsuda, R. Azumi, M. Matsumoto, "A slab-optical-waveguide absorption spectroscopy of Langmuir-Blodgett films with a white light excitation source," in *Chem. Lett.*, Vol. 24, 1995, pp. 437-438.

[3] D. R. Dunphy, S. B. Mendes, S. S. Saavedra, N. R. Armstrong, "The electroactive integrated optical waveguide: ultrasensitive spectroelectrochemistry of submonolayer adsorbates," in *Anal. Chem.*, Vol. 69, 1997, pp. 3086-3094.

[4] K. Tsunoda, T. Umemura, H. Ueno, E. Okuno, H. Akaiwa, "Adsorption of methylene blue onto silylated silica surfaces, studied using visible attenuated total reflection spectroscopy with a slab optical waveguide," in *Appl. Spectrosc.* Vol. 57, 2003, 1273-1277.

[5] N. Matsuda, A. Takatsu, K. Kato, "Absorption spectra of rhodamine 6G by slab optical waveguide spectroscopy," in *Chem. Lett.*, Vol. 25, 1996, pp. 105-106.

[6] K. Kato, A. Takatsu, N. Matsuda, "UV-visible slab optical waveguide spectroscopy of cytochrome *c* adsorbed on a liquid-solid interface," in *Chem. Lett.*, Vol. 28, 1999, pp. 31-32.

[7] Z.-M. Qi, N. Matsuda, A. Takatsu, K. Kato, "A kinetic study of cytochrome *c* adsorption to hydrophilic glass by broad-band, time-resolved optical waveguide spectroscopy," in *J. Phys. Chem. B.* Vol. 107, 2003, pp. 6873-6875.

[8] T. Yoshida, H. Asano, J.H. Santos, Z.-M. Qi, A Takatsu, K. Kato, N. Matsuda, "Studies on adsorption behavior of hemoglobin onto hydrophobic surface by using slab optical waveguide spectroscopy," in *Electronics and Communications in Japan*, vol. J85-C, No. 12, 2003, pp. 1070–1074.

[9] Z.-M. Qi, N. Matsuda, T. Yoshida, H. Asano, A. Takatsu, K. Kato, "Optical waveguide spectrometer based on thin-film glass plates," in *Opt. Lett.* Vol. 27, No. 22, 2002, pp. 2001-2003.

[10] J.H. Santos, N. Matsuda, Z.-M. Qi, T. Yoshida, A. Takatsu, K. Kato, "Adsorption behavior of cytochrome *c*, myoglobin and hemoglobin in a quartz surface probed using slab optical waveguide (SOWG) spectroscopy ," in *Anal. Sci.* Vol. 19, 2003, pp. 199-204.

[11] N. Matsuda, J.H. Santos, A. Takatsu, K. Kato, "Spectroelectrochemical studies on surface immobilized cytochrome *c* on ITO electrode by slab optical waveguide spectroscopy," in *Thin Solid Films* Vol. 438-439, 2003, pp. 403-406.

[12] K. Fukuda, H. Ohno, "Electron transfer reaction of cytochrome c at the electrode surface analyzed with non-contact optical waveguide spectroscopy," in *Electroanalysis* Vol. 14, 2002, pp. 605-610.

[13] K. Fujita, H. Ohno, "Redox reaction of PEO-modified cytochrome c adsorbed on the electrode in ion conductive PEO matrix analyzed with non-contact optical waveguide spectroscopy," in *Polym. Adv. Technol.* Vol. 14, 2003, pp. 486-491.

[14] L. Bedouet, H. Adenier, S. Pulvin, C. Bedel-Cloutour, D. Thomas, "Recovery of the oxidative activity of caged bovine haemoglobin after UV photolysis," in *Biochem. Biophys. Res. Commun.* Vol. 320, 2004, pp. 939-944.

[15] X. Liu, L. Shang, Z. Sun, G. Li, "Direct electrochemistry of hemoglobin in dimethyldioctadecyl ammonium bromide film and its electrocatalysis to nitric oxide," in *J. Biochem. Biophys. Methods*, Vol. 62, 2005, pp. 143-151.

[16] R. Deslauriers, D. J. Moffatt, I. C. P. Smith, "Oxygen consumption in Plasmodium berghei-infected murine red cells: a direct spectrophotomeric assay in intact erythrocytes," in *Biochimica et Biophysica Acta* Vol. 886, 1986, pp. 319-326.

[17] C. Causserand Y. Kara, P. Aimar, "Protein fractionation using selective adsorption on clay surface before filtration," in *J. Membr. Sci.* Vol. 186, 2001, pp. 165-181.

[18] A.-E. F. Nassar, J. F. Rusling, N. Nakashima, "Electron transfer between electrodes and heme proteins in protein-DNA films," in *J. Am. Chem. Soc.* Vol. 118, 1996, pp. 3043-3044.

[19] Y. Liu, H. Liu, N. Hu "Core-shell nanocluster films of hemoglobin and clay nanoparticle: direct electrochemistry and electrocatalysis," in *Biophys. Chemist.* Vol. 117,, 2005, pp. 27-37.

[20] Z. Dai, S. Liu, H. Ju, H. Chen, "Direct electron transfer and enzymatic activity of hemoglobin in a hexagonal mesoporous silica matrix," in *Biosens. Bioelectron.* Vol. 19, 2004, pp. 861-867.

[21] H.-Y. Gu, A.-M. Yu, H.-Y. Chen, "Direct electron transfer and characterization of hemoglobin immobilized on a Au colloid-cysteamine-modified gold electrode," in *J. Electroanal. Chem.* Vol. 516, 2001, pp. 119-126.

[22] A. Kondo, S. Oku, F. Murakami, K. Higashitani "Conformational changes in protein molecules upon adsorption on ultrafine particles," in *Colloids Surf. B: Biointerf.* Vol. 1, 1993, pp. 197-201.

[23] A. Kondo, J. Mihara "Comparison of adsorption and conformation of hemoglobin and myoglobin on various inorganic ultrafine particles," in *J. Colloid Interface Sci.* Vol. 177, 1996, pp. 214-221.

[24] B.A. McCool, R. Cashon, G. Karles, W.J. DeSisto, "Silica encapsulated hemoglobin and myoglobin powders prepared by an aqueous fast-freezing technique ," in *J. Non-Cryst. solids* Vol. 333, 2004, pp. 143-149.

[25] J. Chen, J.D. Andrade, R.A. VanWagenen, "Oxy-and deoxyhaemoglobin adsorption onto glass and polymer surfaces," in *Biomaterials* Vol. 6, 1985, pp. 231-236.

[26] R. F H. FREEMAN, "Haemoglobin in the digenetic trematode proctoeces subtenuis (linton)," in *Comp. Biochem. Physiol.* Vol. 10, 1963, pp. 253-256.

[27] H-Y. Chen, Y-T. Long, "Study of biomolecules by combining electrochemistry with UV/Vis, IR and surface enhanced Raman scattering spectroscopy by a novel flow miccrocell," in *Anal. Chem. Acta* Vol. 382, 1999, pp. 171-177.