UV-visible Slab Optical Waveguide Spectroscopy of Cytochrome *c* Adsorbed on a Liquid-Solid Interface

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A slab optical waveguide spectroscopy (SOWG spectroscopy) was applied to the UV-visible absorption spectroscopy. The SOWG with quartz core layer and amorphous fluorocarbon polymer clad layer has good transparency in UV-visible light region. It was demonstrated that adsorption process on the SOWG surface of cytochrome *c* was successfully observed in situ.

We have reported slab optical waveguide spectroscopy (SOWG spectroscopy) which is a kind of internal reflection spectroscopy. SOWG is a very thin internal reflection element.[1,2] This method is selectively sensitive to interface molecules. In situ observation of adsorption process of dye molecules[3-5] and measurement of visible spectra of monolayer such as LB films[6] was reported. Spectroscopic method has been frequently applied to characterize immobilized protein monolayers,[7] because surface immobilized protein systems have been widely used for enzyme reactor, biosensor, and affinity chromatography recently. The slab optical waveguide spectroscopy has aroused strong interest in the study of proteins,[8] because of the sensitivity advantage to surface thin film. The light absorption bands of proteins exist from UV to visible region. However, with the conventional SOWG equipment, measurements were limited to the wavelength region longer than 400 nm. A major factor of limitation was that proper SOWG was not available in the UV region. Moreover, the light loss in UV region in the optics such as optical fibers, coupling prisms and coupling liquid was another reason of the limitation. In this study, a slab optical waveguide spectrometer is developed to extend its wavelength area to UV region, and light absorption spectra of cytochrome *c* adsorbed on the SOWG surface is measured.

The SOWG for UV spectroscopy made in our laboratory is three layered and is schematically shown in Figure 1. The core is thin quartz glass plate (100 μm thickness, Shin-Etsu Quartz Products, Suprasil-P). The backside of the core was coated with amorphous fluorocarbon polymer as a clad. This amorphous fluorocarbon polymer has features suitable for material of cladding that it is transparent in wide wavelength region (UV to NIR) and has low refractive index of 1.34. This layer is made from a solution of fluorocarbon polymer (Asahi Glass, Cytop CTX-809S). These two layers are fixed on the glass substrate (25 × 75 mm, 1 mm thick) with the glue of the epoxy resin. This SOWG was robust and the handling was easy. The equipment was made in our laboratory and was similar to that used in previous studies.[3,4,6] In order to measure UV spectra, modification were made in spectrometer, light source, coupling prisms, coupling liquid and arrangement of the optics. The spectrometer for visible region was replaced by a 25-cm spectrometer (Chromex, 250i) with a UV-sensitive CCD detector. The pulsed Xe lamp (150 mJ/pulse, maximum repetition rate 100 Hz, Hamamatsu Photonics, L2358) was use as the light source. An ordinary Xe lamp (500 W, USHIO UXL-500D) was used for comparison. In case of the ordinary Xe lamp, the light is transported and introduced using UV-grade optical fiber, UV-grade objective lens and a sapphire coupling prism. The pulsed Xe lamp was small and could be placed near the slab optical waveguide. Therefore, the light was directly introduced into the SOWG by using a single quartz lens without using optical fiber or objective lens. Glycerin-water mixture was used as coupling liquid. In the spectra measurement of cytochrome *c*, phosphate buffer (pH=7, 200 μL) was placed on the core of SOWG and was measured at first as a blank. Then buffer solution was removed and the buffered sample solution (cytochrome *c*, pH=7, 51 μmol/L, 200 μL) was measured. The length of the area where the SOWG contacts with the sample solution was fixed to 2 cm. In the measurement of time-course of

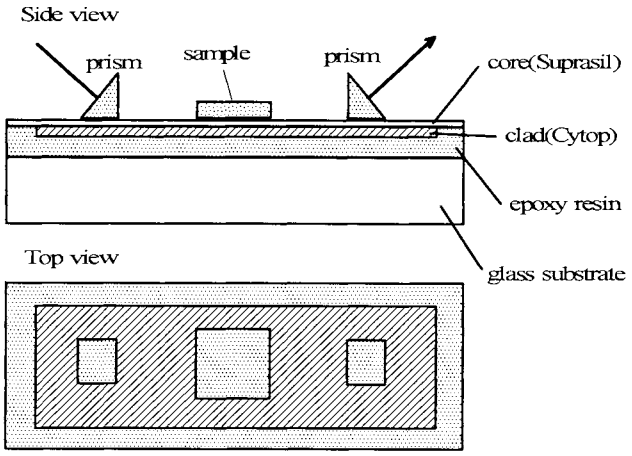

Figure 1. Schematic of the slab optical waveguide structure.

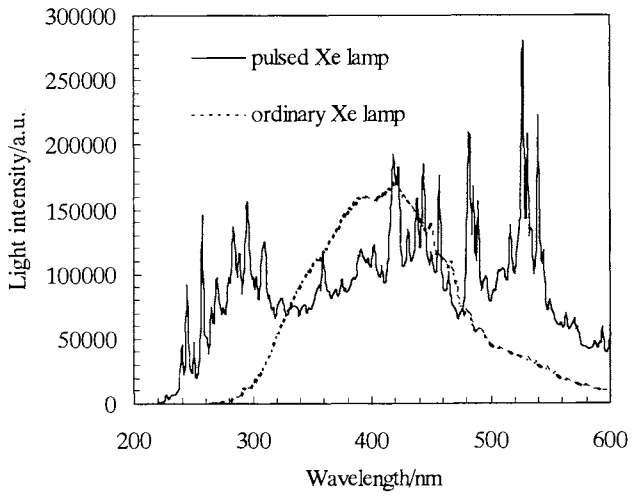

Figure 2. Blank spectra of buffer solution using an ordinary and a pulsed Xe lamps.

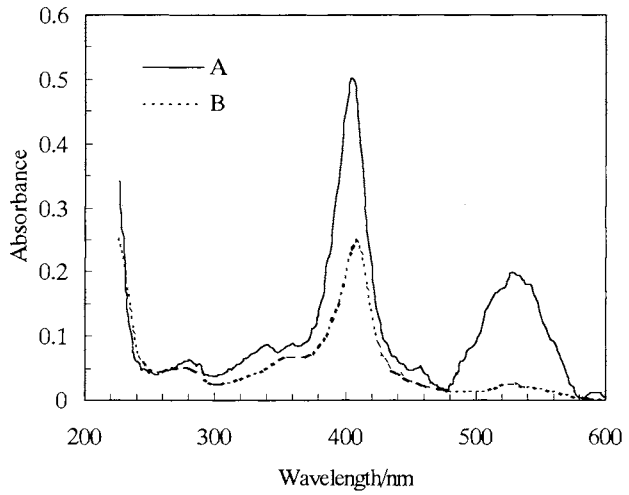

Figure 3. UV-visible spectrum of cytochrome *c* adsorbed on an interface between a buffer solution and an SOWG surface (A) and UV-visible spectrum of the cytochrome *c* solution measured by an ordinary spectrophotometer using a 1cm cell (B).

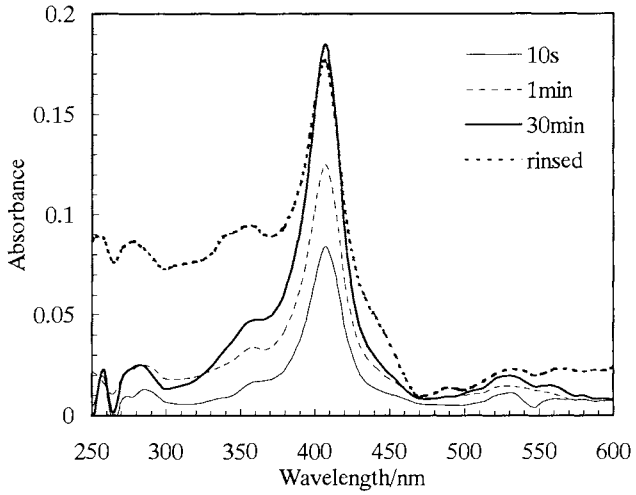

Figure 4. Time dependent change of absorption spectra of cytochrome *c* (in-situ measurement).

spectra, phosphate buffer (pH = 7, 200 μL) on the core of SOWG was measured at first as a blank. Then the buffered sample solution (cytochrome *c*, pH = 7, 51 μmol/L, 10 μL) was added to the buffer solution on the SOWG and a light absorption spectra were measured in the constant time interval. The concentration of cytochrome *c* was about 2.4 μmol/L. Cytochrome *c* was purchased (horse heart origin, Sigma Company) and used as obtained.

The transparency of SOWG in the UV region was satisfactory and transmittance at 230 nm was 80% (ratio to the maximal transmittance at 450 nm). Figure 2 shows the blank spectra using the ordinary xenon lamp and the pulsed xenon lamp. The light intensity of the pulsed Xe lamp was very strong in UV region compared with the conventional Xe lamp. The wavelength where the light intensity becomes twice as large as the background was 260 nm for ordinary Xe lamp, and that for the pulsed Xe lamp was 220 nm. A lot of line spectra were found in the blank spectra of the pulsed xenon lamp. These line spectra could be cancelled by taking the ratio of the sample and the blank spectra. When the pulsed Xe lamp was used, spectrum measurement was completed within a period for 1 to 10 light pulses. This is another advantage of the pulsed Xe lamp, because it prevents sample from damage caused by UV irradiation. Therefore, UV-visible spectrum down to 227 nm became possible by the use of a pulsed Xe lamp as shown in Figure 3 (line A). Strong absorption bands are observed at <230, 405 and 530 nm. This spectral pattern corresponds to the absorption spectrum of oxidized form cytochrome *c* solution measured by a conventional spectrophotometer (Shimadzu, UV-260) with an ordinary liquid cell with light path length of 1 cm (Figure 3, line B).

The time dependence of the absorption spectrum after adding cytochrome *c* to the buffer solution (blank) is shown in Figure 4. The absorbance of the peak at 405 nm became larger with the time and this showed the process of adsorption of molecules on the SOWG surface. The buffer solution containing cytochrome *c* was removed and the SOWG surface was rinsed with the buffer solution after 90 min. Then the absorption spectrum (line noted as "rinsed" in Figure 4) was measured with a new buffer solution. The spectrum was approximately identical to that measured before the SOWG surface was rinsed. This shows that cytochrome *c* molecules were immobilized on the SOWG surface. However, the absorption in the short wavelength region increased and there is a possibility that protein molecule layer was denatured in exposing to the air. In summary, we developed a UV-visible (from 220 nm) SOWG and demonstrated the possibility of the slab optical waveguide spectroscopy in studies of adsorption phenomena in protein solutions.

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