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Talanta 65 (2005) 1066–1070

Talanta

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Dynamic analysis of aggregation of methylene blue with polarized optical waveguide spectroscopy

Kyoko Fujita, Keisuke Taniguchi, Hiroyuki Ohno*

Department of Biotechnology, Tokyo University of Agriculture and Technology, Koganei, Tokyo 184-8588, Japan

Received 19 November 2003; received in revised form 29 March 2004; accepted 16 April 2004
Available online 28 November 2004

Abstract

Polarized optical waveguide (POW) spectroscopy permits analysis of the changing molecular state of methylene blue (MB), including aggregate order and orientation at the waveguide surface. Monomer or dimer, dissolved randomly in MB aqueous solution, tends to aggregate at the waveguide surface during air drying. Furthermore, POW spectroscopy dynamically revealed that MB molecules became oriented vertically to the waveguide surface with increasing aggregation order.

Keywords: Evanescent wave; Orientation; Aggregation; Methylene blue; Dynamics

1. Introduction

Optical waveguide (OWG) spectroscopy is a technique based on total internal reflection. OWG spectroscopy is a highly sensitive method of detecting molecules from the absorption of the evanescent wave emerging from the waveguide surface [1,2]. The intensity of the evanescent wave declines exponentially with distance from the surface. The characteristic distance for this decay is related to the wavelength of the incident light. For visible light, it is several hundred nanometers [3,4]. The incident light propagates by repeated total reflection in the waveguide, and the evanescent wave generated at every reflection is absorbed by samples on the waveguide. The accumulation of absorbed light allows the absorption spectra of the samples to be detected with reliable signal to noise ratio. The OWG technique has already been used in various studies [5–7]. For this, thinner waveguides are preferred for more frequent internal reflections.

By using polarized incident light, OWG spectroscopy is able to analyze the orientation of molecules [8]. Saavedra and co-workers have reported the tilt angle of phthalocyanine bilayers on the waveguide [8]. Analysis of the orientation of molecules at the surface is essential in studying the dynamic behavior of certain molecules using advanced measuring techniques. Orientational analysis with OWG spectroscopy uses two kinds of polarized light, as shown in Fig. 1. Polarization of incident light was set either to transverse magnetic mode (TM) which is perpendicular to the waveguide or to transverse electric mode (TE) which is parallel to the waveguide. When the molecular absorption moment is aligned with the constituent polarized light, the incident light is absorbed by the molecules. For example, when the molecules are lying with those molecular absorption moments parallel to the waveguide (as shown in Fig. 1), TE polarized light is absorbed by this molecule (b), but no absorption of TM polarized light is expected to take place (a). Furthermore, even when the molecular absorption moment is aligned with the direction of polarized light (c), TE polarized light will scarcely be absorbed by this molecule [9]. A formula gives the tilt angle of the molecules from the observations [10]. This requires the dichroic ratio (I_{TE}/I_{TM}) for the absorbance of TE and TM polarized light, and the refractive index of the molecules and the waveguide. In OWG spectroscopy, the definition of the absorbance obtained from the spectrum is different from that in ordinary UV–vis absorption spectroscopy. The absorption

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[*] Corresponding author. Tel.: +81 42 388 7024; fax: +81 42 388 7024.
E-mail address: ohnoh@cc.tuat.ac.jp (H. Ohno).

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doi:10.1016/j.talanta.2004.04.044

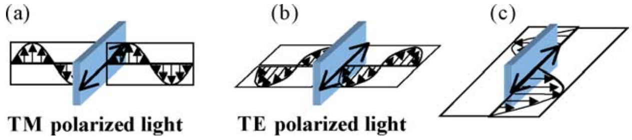

Fig. 1. Scheme of the polarized light mode and molecular absorption moment.

observed in the present study is a function of the penetration depth and intensity of the evanescent wave and the number of reflections. There are many quantitative studies of OWG spectroscopy [7,11], but the term “absorbance” used in this paper means the quantity of absorption observed by OWG spectroscopy. In the present study we investigate the orientation of methylene blue (MB) at the waveguide surface by polarized optical waveguide spectroscopy. Many optical studies have performed on MB in solution [12,13]. Previous studies found that MB molecules existed as dimer or as aggregates at the surface, as well as a protonated form depending on the concentration and the surface properties [14–19]. These reports mentioned the orientation of aggregates on the substrate based on visible spectral data (Table 1). However, there is no report on the dynamics of the adsorption process of molecules onto the substrate. In this study we present a dynamic analysis of the molecular orientation of MB in the adsorption process on the quartz substrate, and changes of orientation during air drying of the MB solution.

2. Experimental

Methylene blue (MB) was purchased from Merck and used without further purification. Water was purified by reverse osmosis dialysis (Milli-Q SP system) to have specific resistance greater than 18.3 MΩ/cm. Absorption spectra of MB were obtained in the wavelength range of 300–700 nm using an OWG spectrophotometer (SIS-50, System Instruments Inc.) [20]. A 150 W xenon lamp (Hamamatsu Photonics) was used as a light source. A light beam was modulated by a mechanical chopper, introduced into an optical fiber through a microscope object lens, and focused at the edge of the quartz waveguide. After propagating through the waveguide, the transmitted light was collected by a second micro-lens attached to another optical fiber, and was directed to a multichromator equipped with photodiode arrays.

As an optical waveguide, a quartz plate (Shin-Etsu Quartz Products, 65 mm × 20 mm) was used, having thickness of 200 μm and refractive index, 1.46. This OWG plate is well suited to analysis in the visible light region, since it has almost 100% transmittance between wavelengths 300 and 700 nm. The waveguide was fixed on a stage that could be moved in any direction. The incident light angle was set at 73.5°. The average number of reflections in this waveguide (65 mm) is then estimated to be 47. The incident light was polarized by a Glan-Thompson polarizing prism placed between the waveguide and the incident optical fiber. The samples prepared on the waveguide were analyzed using two incident polarized light beams (TE and TM polarized light) [21]. MB was dissolved in milli-Q water at 0.1 mM concentration. This solution was poured directly onto the waveguide (100 μl/cm^2) and OWG spectra were immediately recorded. The same 0.1 mM MB solution also underwent POW measurement and ordinal UV–vis absorption spectrum analysis (in a quartz cell with an optical path length of 10 mm). For POW spectroscopy the MB layer was prepared by air drying of the MB solution poured onto the waveguide. The effect of differing concentrations of MB solution on the molecular state in the cast layer was analyzed using 0.1 and 4.5 mM solutions. These concentrations were chosen by consideration of the concentration of MB for dimer formation (0.2 mM) in aqueous solution [22]. After pouring of the 4.5 mM MB solution onto the waveguide, spectral changes deriving from changes in molecular orientation and aggregation during drying were investigated using TE and TM polarized light.

Table 1
Absorption maxima (λ_{max}/nm) of MB molecules

Assignment	Dissolved in water[a]	Adsorbed on smectites in an aqueous phase[a]	Cast layer on quartz plate[b]
Monomer	662	653, 670	640, 670
Monomer and dimer	610	600–610	600
Trimer and aggregates	n.d.	570	570
Higher aggregates	n.d.	n.d.	545

n.d.: not detected.
[a] Refs. [15,18].
[b] This study.

3. Results and discussion

The absorption moment of the MB molecule is along its long axis, as seen in Fig. 2. Increasing the concentration of the MB aqueous solution is known to cause a blue shift of the major absorption band due to H-aggregation [19]. Fig. 2(a) shows the OWG spectrum of 0.1 mM MB aqueous solution

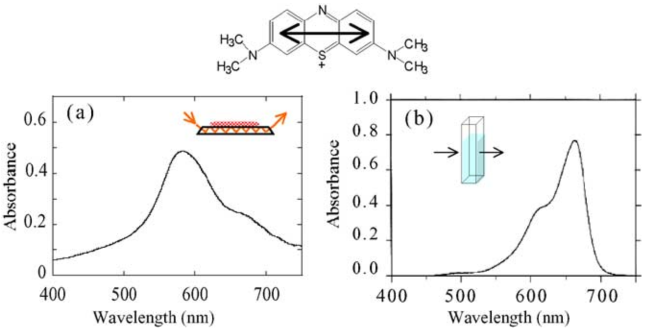

Fig. 2. Absorption spectra of MB solution observed by OWG spectroscopy (a) and ordinary UV–vis spectroscopy (b).

poured onto the waveguide. The spectrum with λ_{max} at around 590 nm is attributable to the dimer or to slightly higher aggregates of MB molecules. When the concentration of the MB solution was increased from 0.05 to 5 mM, the absorbance of the OWG spectrum became greater but the spectral shift at λ_{max} was barely detectable (data not shown). With an ordinary UV–vis absorption spectrum of 0.1 mM MB aqueous solution, λ_{max} was detected at 660 nm, suggesting a monomeric state (Fig. 2(b)). These results show that MB molecules are dissolved in a monomeric state in aqueous solution, but that they form aggregates at the quartz surface. After the 0.1 mM MB aqueous solution was poured onto the waveguide, the resulting layer was analyzed with TE and TM polarized light. There was no significant difference in intensity or in λ_{max} between the two spectra detected with these polarizations. Based on these results, we suggest that the homogeneously dissolved MB molecules form dimer or low aggregates near the quartz surface, but that these aggregates are without specific orientation.

Fig. 3 shows the POW spectra of the cast MB layer (solid phase) on the waveguide. When the layer was cast from 0.1 mM MB aqueous solution by air drying, TE and TM POW spectra both showed three peaks at 600, 640, and 670 nm (Fig. 3(a)). These peaks correspond to monomer, dimer and a low aggregate of the MB molecules. Absorption of TE polarized light was about twice than that of TM polarized light. This difference suggests that the direction of the absorption moment of monomer or lower aggregate MB is almost parallel to the waveguide surface. With TM polarized light, absorption peaks were observed at 540 and 570 nm that were not seen with TE polarized light. H-aggregation represented by MB molecules shows a blue shift of the major absorption band with the formation of higher aggregates [23]. When infinite molecular aggregation was assumed, λ_{max} was calculated to be around 540 nm based on Kasha's theory [24]. This theoretical value agrees well with the observed shorter wavelength peak, which is therefore attributed to highly aggregated MB. This absorption peak at 540 nm was observed only when TM polarized light was used. The direction of the absorption moment of highly aggregated MB molecules should therefore be nearly perpendicular to the waveguide surface, so that the MB molecules are standing on the waveguide. These results suggest that MB molecules aggregate randomly in the air-dried cast MB layer from 0.1 mM MB aqueous solution.

The MB layer cast by air drying of the 4.5 mM MB aqueous solution was also analyzed with polarized light. The spectra observed were very different, as seen in Fig. 3(b). With TM polarized light, λ_{max} was observed at 545 nm, suggesting that MB molecules form large aggregates standing on the waveguide. With TE polarized light, by contrast, a broad peak was observed around 620 nm. This suggests that MB molecules lying on the waveguide were almost monomeric. A single peak was observed with both polarizations, and no other characteristic peaks were observed. In the MB layer cast by air drying of 4.5 mM aqueous MB solution, MB molecules appear to exist in two types. One is a highly aggregated state standing up on the waveguide surface, and the other is a monomer state lying on the waveguide (Fig. 3(c)). The values of λ_{max} for MB species at differing degrees of aggregation were compared. The values of λ_{max} observed by OWG spectroscopy for every aggregated state agree well with those for MB molecules adsorbed on smectites in aqueous solution. The absorption observed at 545 nm following air drying of 4.5 mM MB solution suggests the presence of highly aggregated MB molecules. The aggregation order of MB in this cast layer should be higher than that adsorbed on smectites or in aqueous solution.

To discuss the dynamic adsorption process of MB during the air drying process, POW spectra were measured. Fig. 4 shows the dynamic change of both λ_{max} and the absorbance at λ_{max} during air drying. When 4.5 mM aqueous solution was poured onto the waveguide, λ_{max} was at 600 nm by TM polarized light (Fig. 4(a)). A slow increase in the absorbance was then observed, but little shift in λ_{max}. Thereafter, λ_{max} showed a considerable blue shift with an increase in absorbance. The MB solution was concentrated by drying, and some aggregation then occurred. Strong absorption at 545 nm was observed with TM polarized light. This strongly suggests that the MB molecules were highly aggregated, and were standing vertically to the waveguide. When spectral changes were analyzed with TE polarized light, a λ_{max} value around 600 nm was observed immediately after pouring of the MB aqueous solution onto the waveguide. The absorbance at 600 nm was then ob-

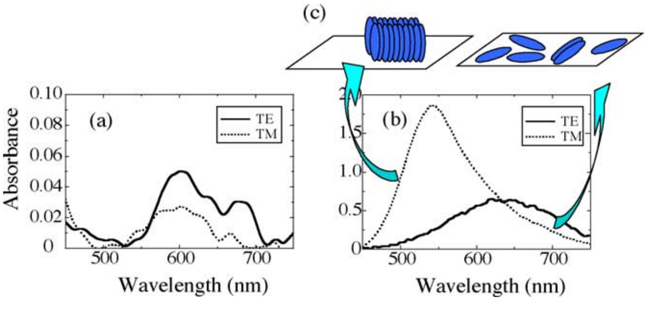

Fig. 3. POW spectra of MB layer cast by air drying of 0.1 mM (a) and 4.5 mM (b) MB aqueous solutions. (c) Scheme of estimated state of MB on the waveguide.

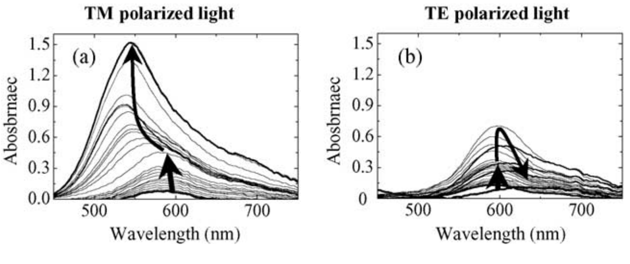

Fig. 4. Dynamic change of MB spectra during air drying observed by TM polarized light (a) and TE polarized light (b).

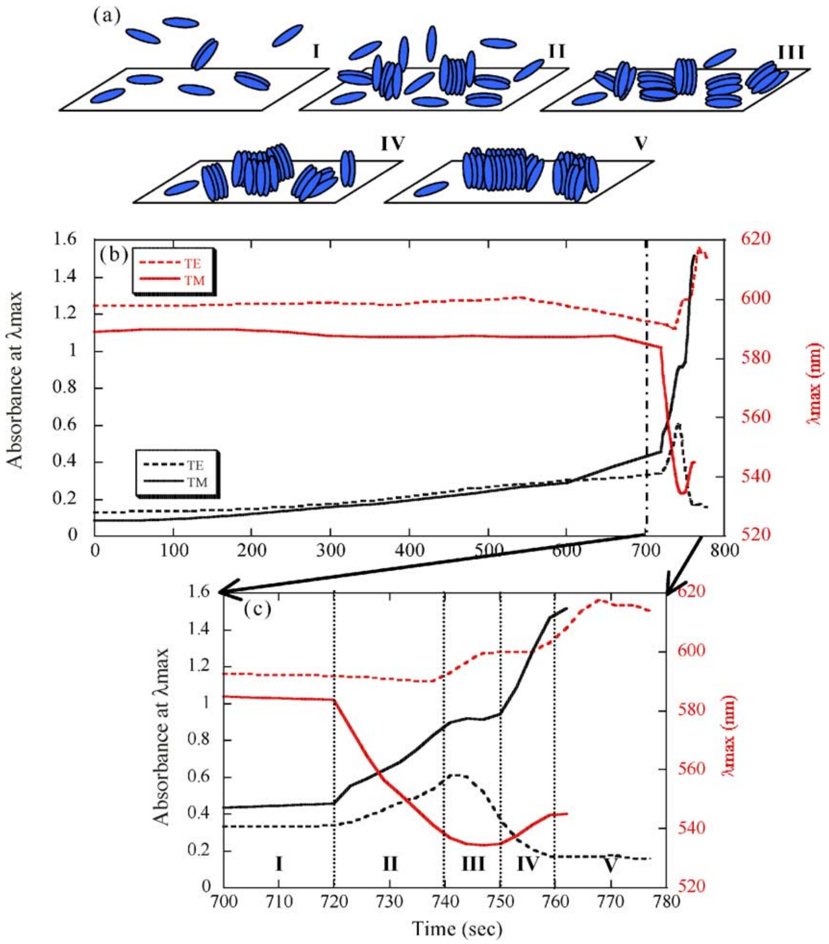

Fig. 5. (a) Estimated molecular state of MB. (b) Changes of λ_{max} and the absorbance at the λ_{max}. (c) Changes of the previous parameters at the final part of (b).

served to increase slowly for a while, as seen in Fig. 4(b). This increase was also explained as an increase in the concentration of MB molecules with drying. However, a rapid fall in the absorbance was then observed, followed by a red shift. The final value was 620 nm. Fig. 5(b) shows the changes of λ_{max} and the absorbance at λ_{max}. Steep spectral changes are clearly seen just before complete drying with both TE and TM polarized light. Before that, there are slow increases in absorbance, presumably due to an increase in concentration. Fig. 5(c) shows a magnification of the final part of Fig. 5(b), just before complete drying. Fig. 5(a) shows a schematic illustration of the molecular state suggested by these dynamic changes in the spectra. Changes in the molecular states were analyzed through changes of λ_{max} and the absorbance with time. In this experiment, the change in absorbance with increasing concentration was accelerated at around 720 s after the MB aqueous solution was poured onto the waveguide. The period between 0 and 720 s was classified as phase I. In phase I, a steady increase in the concentration of MB is suggested by data from both TE and TM polarized light. Following phase I, a rapid spectral change occurs that is attributed to self-interaction of MB or interaction of MB with the waveguide surface within the penetration depth of the evanescent wave. In phase II (720–740 s), the increase in absorbance was further accelerated under both TE and TM polarized light. However, the TE polarized spectrum showed no shift of λ_{max} at 600 nm. This suggests that aggregations of few molecules (mostly dimer) were still randomly dissolved in the solution. In fact a blue shift of λ_{max} was observed with increasing absorbance using TM polarized light. We suggest that the extent of aggregation of MB molecules progresses with the increase of the fraction of standing MB molecules. Since the shift of λ_{max} was observed only when TM polarization light was used, the dissolved MB molecules appear to form aggregates standing on the waveguide. After phase II, the sum of the observed absorbances for TE and TM polarized light increased, tending towards a constant value. This strongly suggests that all MB molecules were by then concentrated near the waveguide, and especially within the range that the evanescent wave could reach.

In phase III (740–750 s), a significant spectral change was observed in the TE polarized light system. The absorbance now decreased followed by a red shift of λ_{max}. From these changes, it appears that the fraction of lower aggregates lying on the waveguide decreases, and the remaining monomers were lying there. Further aggregation of lower aggregates should occur, but these were not yet standing on the waveguide, since no increase in absorbance was observed under TM polarized light. At phase IV (750–760 s), where the absorbance of TM polarized light increases again, the aggregated molecules are standing. Finally, almost all the MB molecules are aggregated together and standing on the waveguide, with a small fraction of MB monomers and dimers lying (phase V). The spectral changes seen in Fig. 5 were repeatedly observed upon pouring MB solution onto the waveguide.

4. Conclusions

The aggregation and adsorption behavior of MB molecules has been examined by the POW technique. MB molecules dissolved in aqueous solution exist as dimer or as lower aggregates at the substrate surface; this is a different state from that in the bulk. However, the direction of the absorption moment of these lower aggregates was not oriented, but random. We suggest that, when low concentration MB aqueous solution is cast on the waveguide, there are mixtures of molecularly dispersed MB, dimer, and other lower aggregates. On the other hand, when relatively concentrated solution, greater than the lowest concentration for dimer formation, was used to prepare the cast layer, monomer and dimer existing randomly in the solution were well aggregated in the concentration process. Also, the absorption moment of highly aggregated MB molecules clearly implied that they were standing vertically to the waveguide. Fig. 5 shows the aggregation and orientation changes of MB molecules, analyzed dynamically with time. During continuous air drying of a poured MB aqueous solution, a gradual increase in the concentration of MB molecules in the vicinity of the waveguide was observed. Drastic changes were detected at the final stage of drying. The fraction of dimer or lower aggregates increased, but there was no specific orientation of these aggregates. At the waveguide surface most aggregates are standing, and homogeneously dissolved MB molecules directly participate to form standing aggregates. Before complete drying, most MB molecules existed as aggregates, but only a small fraction of monomeric MB lying on the waveguide surface was observed. POW spectroscopy is an extremely powerful tool for the dynamic analysis of aggregation of dye molecules.

Acknowledgement

The present study was carried out under the 21st COE program of Future Nano-Materials. One of the authors (K.F.) acknowledges the financial support of the Japan Society for the Promotion of Science (Research Fellowships for Young Scientist). The present study was also supported by the Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology, Japan.

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