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Surface-Enhanced Raman Spectroscopic Studies of Oriented Monolayers on Electrode Surfaces

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ABSTRACT : Monolayers of hypericin, a photodynamic polycyclic quinoidal compound, were prepared at the air-water interface, and were transferred to metal substrates to form Langmuir-Blodgett (LB) monolayers. The structural characteristics of hypericin LB monolayers and self-assembled (SA) monolayers were investigated using surface-enhanced resonance Raman scattering (SERRS) spectroscopy. Both the spectroscopic data and the surface pressure - area (π-A) isotherms suggest that hypericin forms π-π aggregates that orient vertically to the subphase surface. Whereas the ordering and orientation of control was less effective in SA monolayers, a higher structural regularity was attained in LB systems. The effect of subphase on the structural integrity of the monolayer was also investigated.

Keywords : surface-enhanced Raman spectroscopy, Langmuir-Blodgett, self-assembly monolayer, hypericin

1. Introduction

Hypericin as shown in *Fig. 1* is a rigid, multi-ring aromatic quinoidal compound that is known to exhibit a variety of photodynamic effects[1,2].

The hypericin is found in certain species of plants such as Saint John's wort[3]. Hypericin and its analogs can be found in the pigment granule of the protozoan *Stentor coeruleus*. *Stentor coeruleus*, a blue-green unicellular ciliate with a size of ca. 400 μm, exhibit photosensitive behavior including a step-up photophobic response and a negative phototaxis in the presence of light[4]. The chromophore of the

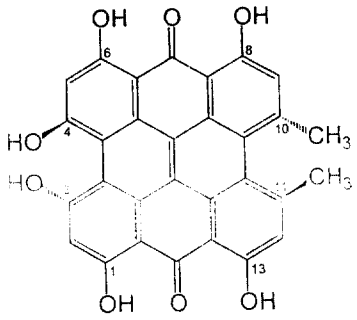

Figure 1. Structure of hypericin.

photoreceptor protein has been determined recently as an analog of hypericin[5]. Recently, hypericin has been demonstrated to be a potential antiviral agent[6]. It was shown to inhibit both the replication and the infection cycles of several retroviruses, such as the Friend leukemia virus (LP-BM5) and the human immumodeficiency virus (HIV)[7]. Several independent studies shown that the presence of light is an important factor for the hypericin's antiviral activity and that target virus should be enclosed with membrane layer. Although the exact origin of the antiviral activity of hypericin is currently not determined, several mechanisms have been proposed. These include superoxide radical formation, singlet oxygen generation and excited state proton transfer[8]. To understand the mechanism of the antiviral activity of hypericin and furthermore, to develop hypericin as an antiviral drug for AIDS treatment, it is essential to understand its chemical and physical characteristics. The preparation of ordered films may be very useful for investigating the specific interaction of hypericin to the lipid layer by construction of LB monolayer of hypericin including lipid molecules as a simplified model of the *in vivo* structure. Raman excitation profiles may yield important information regarding the excited state, which is an critical for the photodynamic effects of the molecule. The focus of this study is to investigate the structural characteristics and the feasibility of the preparation of stable and reproducible LB and SA monolayers of hypericin to solid substrates.

2. Experimental

Hypericin (LC Service Co., Woburn, MA) was spread from a chloroform/methanol (9:1) solution with concentration 0.06 mg/mL. The Langmuir trough, water purification procedures, and details of the sample preparation have been described previously[9]. In present work, the monolayer was compressed at the rate of 7 cm^2/min. The LB films used for the present Raman study were deposited by vertical withdrawal from the subphase at a surface pressure of 20 mN/m onto the substrates. The transfer rate for the LB films was 1.6 mm/min with a constant transfer ration better than 95 %. SERS substrates were both vacuum deposited Ag island films and electrochemically roughened Ag electrodes. The preparation of substrates were described elsewhere [10]. SERS spectra were collected using a Spex Triplemate spectrometer with a Spex Industries CCD (Model LN1024x256-2) detector cooled -120 C. All spectra were collected in a backscattered geometry and the laser power at the sample was approximately 10 mW. The 457.9 nm (Coherent Ar^+ Inova 70) or 568.2 nm (Coherent Kr^+ Innova 302) laser lines were used as the excitation source. All SERS spectra were collected with the substrates immersed in an optical liquid nitrogen dewar.

3. Results and Discussion

The surface pressure - area isotherms obtained for hypericin were found to be very sensitive to the

concentration of the spreading solution. In order to maintain monomeric form in the solution, methanol and chloroform mixture (1:9 v/v ratio) was used for preparation of spreading solution. Even with 10 % methanol solution, concentrations greater than ca. 2 x 10^{-4} M were found to contain aggregates. For instance, the 5.27 x 10^{-4} M solution contained aggregates, whereas the 1.24 x 10^{-4} M solution did not. For the all solutions that contained aggregates the area/molecule were less than 30 Å^2/molecule. Although there is a substantial difference in the absorption spectra of monomeric and aggregated hypericin , the presence of aggregates could not be determined with the UV-vis absorption spectra of the spreading solutions. A typical isotherm for hypericin on water is shown in *Fig. 2*.

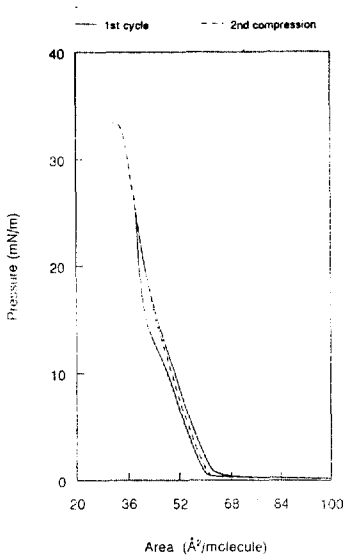

Figure 2. π-A isotherm of hypericin. The subphase was water. The sample solution were 90% chloroform 10% methanol.

The π-A isotherm consists of a simple curve on a pure water subphase, whereas a noticeable plateau at 10 to 15 mN/m pressure range was observed on the subphase containing various ions. The molecular areas obtained by extrapolation back to zero surface pressure were near 58 Å^2/molecule ± 3 Å^2, and were fairly reproducible. Collapse of the molecular films occurred at pressure near 32 mN/m. The hysteresis behavior observed with consecutive compression and expansion cycles is probably the results of strongly associated hypericin molecules at the interface.

Surface-enhanced resonance Raman scattering (SERRS) spectra were obtained for the LB films and compared to those SA films are displayed in *Fig. 3*. Although the spectra feature for the two types of films are for the most part almost identical, the overall spectral intensity for the LB monolayers was 3-4 times greater than that for the SA films. Most of differences seen in the SERRS spectra for the hypericin films are in the 1650 to 1250 cm^{-1} region (the C-C stretching modes of the corner rings). The largest band shifts are seen in the 568.2 nm excitation spectra, where the 1341, 1323 cm^{-1} bands of the SA film is shifted to 1333, 1318 cm^{-1} in the LB film spectra, respectively. In the LB film, the 1250 cm^{-1} band is split into two bands, one at 1255 and another 1242 cm^{-1}. In contrast, most of the band positions for the 457.9 nm excitation spectra are the same for both spectra, although some relative intensity changes are observed. The most notable changes are for the ring bands from 1380 cm^{-1} to 1250 cm^{-1} in the LB film spectrum. These bands are much more intense for the SA film. Relative intensity changes and minor band shifts observed in SERRS spectra are often indicative of orientational differences. The SERRS spectra demonstrate that at least some degree of ordering that is not present in the normal aggregate lattice structure has been obtained through the use of the Langmuir-Blodgett technique.

The orientation of the hypericin at the air water interface in very difficult to discern. Due to lack of the well defined hydrophilic and hydrophobic parts in the molecule, its orientation at the interface is apt to be less regular. The molecular area of hypericin in the plane of the macrocycle obtained from a space filling CPK model is about 12 Å x 12 Å x 5 Å. The areas obtained from the isotherms suggest that hypericin does not orient in a horizontal fashion at the air-water interface. Although the minimum area for hypericin oriented vertically is approximately 60 $Å^2$, the areas obtained on pure water subphase are probably too small for a single monolayer in this configuration. This suggests that there may be some stacking or aggregation in the hypericin films.

Although the exact orientation of the hypericin molecules at the air-water interface can not be determined at this time, a strong case can be made for a strongly-interacting ring stacked structure that is oriented vertically to the water subphase. Three observations support strong interactions between the hypericin molecules in the LB film: 1) the hysteresis observed in the π-A isotherm that was discussed earlier; 2) the strong perturbation of the absorption spectrum; and 3) the LB film was found to be non-fluorescent. Hypericin in a monomeric state is highly fluorescent, but most aggregates are not fluorescent. A π-π stacked structure is supported by the areas obtained from the π-A isotherm.

Other subphases were investigated to determine if better monolayers could be obtained. These include: 1 mM phosphate buffer (pH = 6.5), 10 mM phosphate buffer (pH = 6.5), ~10 mM HCl (pH = 1.3), 10 mM NaOH (pH = 11), 10 mM NaCl, and 10 mM $FeCl_3$. The phosphate buffer and NaCl subphases were chosen to determine the effect of ions on the monolayers,

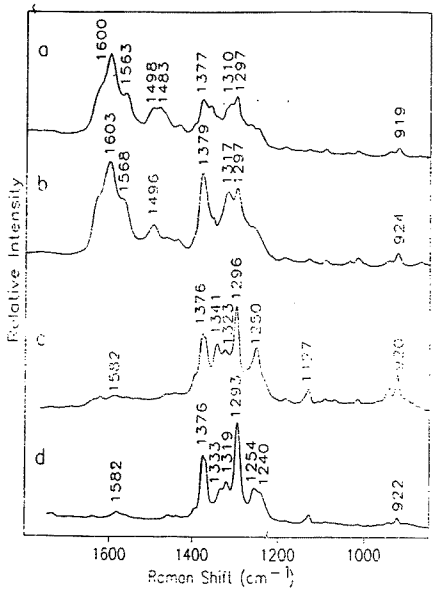

Figure 3. SERRS spectra of hypericin a) SA film at 457.9 nm excitation, b) LB film at 457.9 nm excitation, c) SA film at 568.2 nm excitation, d) LB film at 568.2 nm excitation. Integration times: a and b 100 s, c and d 60 s. 77 K.

whereas the acidic, basic and $FeCl_3$ subphases were selected in an effort to help determine orientations of hypericin at the air-water interface Protons and Fe^{3+} are both known to interact with the carbonyl portions of hypericin[11], whereas OH^- can deprotonate hypericin at position 3[12]. With the exception of $FeCl_3$, all the other subphases gave less reproducible isotherms than those obtained for a pure water subphase. All isotherms displayed phase transitions in the 10-14 mN/m range; however, the length and slope of the transition varied. The isotherm obtained for an $FeCl_3$ subphase were found to be very reproducible with a molecular area of 83 $Å^2$/molecule when extrapolated to zero surface pressure. The area at 30 mN/m was 54 $Å^2$/molecule. This area is somewhat larger than that obtained on a pure water subphase; thus it is possible that hypericin forms a single

oriented layer at the air-$FeCl_3$ containing water interface. The collapse pressure for these monolayers was also higher than for the other films. Hypericin is known to chelate to Fe^{3+} through a carbonyl, so it is reasonable to think that possibly hypericin orients with a carbonyl in the water phase. The band positions in the SERRS spectra (not shown) of the $FeCl_3$ subphase hypericin films were nearly identical to those obtained for the pure water subphase films. However, the spectra for the $FeCl_3$-hypericin films were broader and displayed some relative intensity differences compared to the spectra of the water subphase film. Hydrogen bonding is known to play an important role in both the solvation and aggregation of hypericin. Previous studies have shown that the solvatochromatic behavior of hypericin is related to the hydrogen bonding ability of the solvent[13]. Therefore, any interactions of hypericin at the air-water interface probably would also involve hydrogen bonding. This is supported by the irreproducibility of the films on the NaCl and phosphate buffer subphases. The presence of ions in the subphase likely interferes with the hydrogen bonding interactions at the air-water interface causing the films to become less stable and more susceptible to multi-layer formation. The effects of the acid and base subphase were studied in an effort to gain orientational information. The SERRS spectra obtained for films transferred from the NaOH subphase were identical to the spectra obtained for those from the water subphase at the same transfer pressure. Two possible explanations for this are: 1) the hypericin did not orient at the air-NaOH interface with the position 3 OH close enough to the surface to be deprotonated; or 2) all of the deprotonated hypericin dissolves into the subphase and is not transferred to the SERRS substrate.

The spectra of the films transferred from the acid subphase were different from those obtained from the aqueous subphase. Their SERRS spectra, along with the spectra of SA film formed from acidic hypericin solution (pH = 1), are shown in *Fig.4* for 457.9 nm

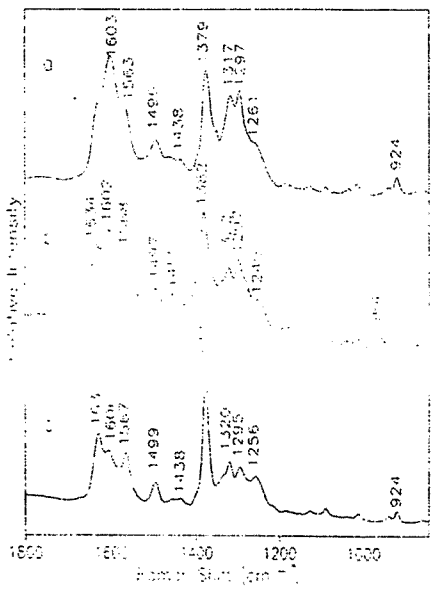

Figure 4. SERRS spectra of hypericin obtained with 458 nm excitation. a) LB film from water subphase, b) SA film from acidic ethanol (pH = 1) hypericin solution, c) LB film from 10 mM HCl subphase. Integration times were 100 s. 77 K.

excitation. In the presence of acid, hypericin is protonated. For the SA film, there are several indicators of protonation that can be observed in the SERRS spectra. One is the enhancement of the shoulder of the 1603 cm^{-1} peak. This band is thought to contain contributions from the hydrogen-bonded carbonyls of the hypericin[14]. When hypericin is protonated, the hydrogen bonding is lost or weakened, which in turn shifts the band to a higher frequency. The band at 1634 cm^{-1} has been assigned to a non-hydrogen-bonded carbonyl of the hypericin. Another indicator of protonation is the large increase in the relative intensity of the 1382 cm^{-1} band. This band has been assigned to the vibration of the central-carbonyl containing fragment of the macrocycle, so some type of change in this band should occur upon protonation of the carbonyl. All of these indicators are also present both in the LB and SA film of hypericin from the HCl subphase. The similarity in SERRS spectra between the SA films from acidic hypericin solution and the LB acid subphase film suggests that there is some interaction of at least one carbonyl of the hypericin at the acid-air interface.

If Fe^{3+} is chelated to a carbonyl of hypericin in the transferred film, several of the same indicators should be observed in the SERRS spectra of the hypericin. The fact that none were observed suggests that the Fe^{3+} does not become incorporated into the transferred films. Although these films were stable at the air-water interface, they do not appear to transfer well. Both the lower transfer ratios and the broader SERRS bands support this. The hypericin films at the air-$FeCl_3$ interface may be too rigid to transfer well. It is not unusual to find that films of rigid molecules do not transfer well with the vertical deposition method.

Conclusions

Monolayers of hypericin at the air-water interface have been studied by π-A isotherms, and have been transferred to solid supports. The spectroscopic data of the transferred films suggests that the films are at least partially ordered. SERRS spectra suggest that there are strong interactions between the hypericin molecules in the LB films. Molecular areas from the π-A isotherms suggest that the ring systems of hypericin are oriented vertically to the subphase but that some multilayer or aggregation may be experienced in the films. Hydrogen bonding is also believed to play an important role for the stabilization of the hypericin films at the air-water interface. However, it is not possible from the present data to determine specifically which functional group(s) are involved in these interactions. From the protonation of the carbonyl observed in the SERRS spectra, hypericin is thought to orient with one of the carbonyls in the acid subphase. A similar orientation may occur at the $FeCl_3$ subphases, however these films did not transfer well. This may be because of their rigidity.

Acknowledgments

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