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Fourier Transform Raman Scattering of Lumichrome Using CW Nd:YAG 1064 nm Excitation

Nam-Soo Lee

Department of Chemistry, Chungbuk National University, Chungbuk 360-763

Resonance Raman spectra of lumichrome[1] as well as lumiflavin, riboflavin, and flavoproteins[2] adsorbed on the colloidal silver surface using Ar^{+} ion laser 488 nm excitation exhibited characteristics of a resonated isoalloxazine ring system. From these findings, it was proposed that the active species of flavin chromophores adsorbed on the metal surface can be a deprotonated form at N_3-H position of the flavin ring III. Recently, Fourier transform(FT) Raman spectra[3] of lumiflavin and riboflavin in solid state using cw Nd:YAG laser 1064 nm excitation far from the electronic absorption bands of flavin chromophores have exhibited characteristics of an isoalloxazine and the carbonyl stretching modes of the flavin ring III. When adsorbed on aqueous colloidal silver metal surface, however, FT Raman and resonance Raman spectra showed only characteristics of a resonated isoalloxazine, but the carbonyl stretching modes of the flavin ring III were completely depressed. These findings were applied to clarify the adsorption behavior on the metal surface of flavin chromophores through two oxygens of $C_4=O$ and $C_2=O$. It can be interpreted, at the same time, as following that the adsorption through two oxygens of the flavin ring III on the metal surface induces a resonated delocalization of isoalloxazine chromophores, possibly *via* a charge transfer mechanism. The anomalous enhancement of Raman scattering even using near infrared excitation far from resonance Raman conditions may be attributed, in part, to this sort of chemical mechanism.

Lumichrome (7,8-dimethylalloxazine), one of the species of flavin-like molecules, has not received much attention so far in spectroscopic fields of Raman or NMR and in normal coordinate calculation study. The structure, the atomic numbering and ring numbering of lumichrome are drawn in Figure 1(a) and 1(b). It does not retain a methyl group at N_{10} position of lumiflavin. The comprehensive normal coordinate calculation studies for lumiflavin were accomplished by several groups.[4-6] Absorption spectrum of lumichrome, with pale yellow color, in the visible range is moderately different from that of lumiflavin, riboflavin or flavin mononucleotide with deep yellow color. The electronic absorption maximum of lumichrome occurs at near 390 nm, but near 450 nm for isoalloxazine chromophores, slightly varying with different solvents and concentrations.

In this report, we present FT Raman spectrum of lumichrome to reveal the details of the spectroscopic differences from the isoalloxazine chromophores. These data would be applied to explore the probable structural changes when lumichrome is adsorbed on the colloidal silver metal surface. FT-IR and FT Raman spectra of lumichrome in solid phase have been shown in Figures 2(a) and 2(b) in the range of
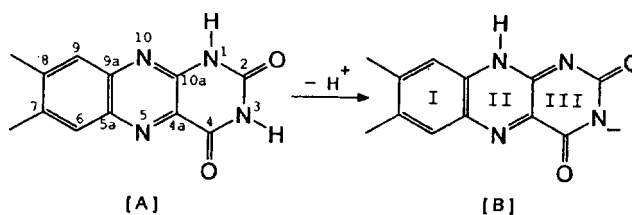

Figure 1. (a) The structure and atomic numbering (1, 2, 3,···) of lumichrome, (b) Ring numbering (I, II, III) and the probable structure when adsorbed on the colloidal silver metal surface.
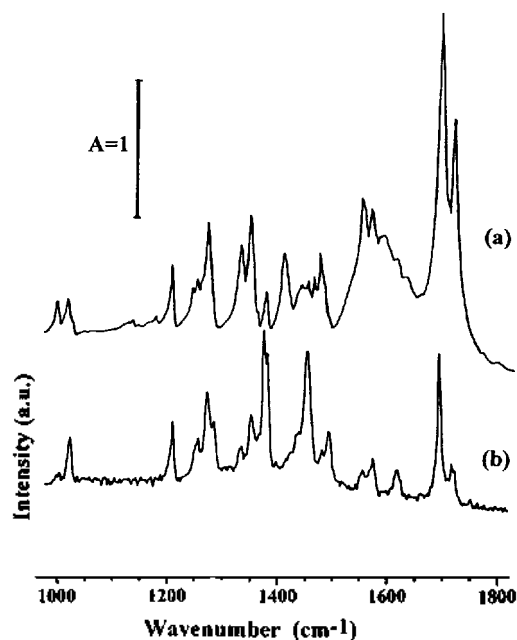

Figure 2. (a) FT-IR spectrum of lumichrome in solid phase at spectral resolution 4 cm^{-1}, coadding 100 times, *in vacuum* mode recorded using a Bomen DA 3.002 spectrophotometer equipped with a liquid Nitrogen-cooled MCT detector, a globar light source, and a KBr beam splitter (b) FT Raman spectrum of lumichrome in solid phase at spectral resolution 4 cm^{-1}, coadding 1000 times, laser power 0.1 W at sample, recorded using the same machine as above but equipped with liquid nitrogen-cooled InGaAs detector, 1064 nm excitation source from a Quantronix continuous-wave Nd:YAG laser, a quartz beam splitter, and a back-scattering collection optics system. (A: Absorbance, a.u.: arbitrary unit)

1000 to 1800 cm^{-1}, respectively. FT-IR spectrum obtained is basically the same as dispersive IR spectrum[7] appeared earlier, but here displayed in the absorbance scale. Their vibrational bands are summarized in Table 1 and tentatively assigned to collate with those of surface enhanced Raman spectrum[1] of 1.0 μM lumichrome in silver hydrosol.

Figures 2(a) and 2(b) have a strong band at 1697 cm^{-1} and a medium size band at 1723 cm^{-1} in the carbonyl stretching region. A band at 1723 cm^{-1}, due primarily to contribution from v ($C_4=O$), was observed as a doublet with a band at 1718 cm^{-1} as seen in the FT Raman spectrum[3] of lumiflavin. This doublet was considered to be the Fermi resonance of the $C_4=O$ stretching mode with the combination

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Table 1. Observed FT Raman and FT-IR Frequencies (cm⁻¹) of Lumichrome in solid state and SERS Frequencies

Band	Raman	FT-IR	SERS*	Assignments**
1	1723	1725	(no)*	amide I, ν (C_4=O)
1'	1718	sh⁺	(no)	
2	1697	1700	(no)	amide I, ν (C_2=O)
3	vw⁺⁻	1641	(no)	
4	1620	1623	(1629)	ν (C_8-C_9), ν (C_{5a}-C_{9a})
5	vw	1598	(no)	
6	1576	1578	(1573)	ν (N_{10}-C_{10a}), ν (N_1-C_{10a})
7	1558	1561	(1548)	ν (C_{4a}-N_5), ν (N_1-C_{10a})
8	1496	vw	(1525)	ring I, II
9	1484	1484	(1481)	ring I, II
10	sh	1471	(sh)	δ^{as} (CH_3)
11	1458	1461	(1460)	ν (C_7-C_8)
12	1441	1447	(no)	δ (N_1-H)
13	sh	1420	(no)	δ (N_3-H)
14	1386	1384	(1377)	ν (C_{10a}-N_1), ν (C_4-N_3)
15	1380	vw	(1377)	ν (C_{10a}-N_1), ν (N_5-C_{5a})
16	1356	1357	(1348)	ν (C_{5a}-C_6), ν (N_{10}-C_{9a})
17	vw	1339	(1322)	
18	1288	vw	(no)	ν (C_{4a}-C_{10a})
19	1279	1281	(no)	amide III, ν (C_4-N_3)
20	1261	1261	(no)	
21	sh	1253	(no)	
22	1213	1214	(1212)	ν (C_4-N_3), ν (C_4-C_{4a})
23	vw	1185	(1185)	
24	vw	1144	(1155)	
25	1026	1024	(no)	δ (C_9-H), δ (C_6-H)
26	vw	1006	(no)	

*from reference 1, **tentatively assigned from reference 4, 5 and 6. (ν: in-plane stretching, δ: in-plane bending, δ^{as}: anti-symmetric deformation), * not observed, ⁺ shoulder, ⁺⁺ very weak intensity.

modes of the vibrations including the N_3-H deformation mode.[5] When compared to corresponding bands (1660 cm⁻¹ and 1716 cm⁻¹)[3] of lumiflavin, a band at 1697 cm⁻¹, due mainly to contribution from ν (C_2=O), is upward shifted as much as 37 cm⁻¹ and gains a strong intensity, but a band at 1723 cm⁻¹ is only 7 cm⁻¹ upward shifted. These observations strongly indicate that the conjugation effect at the bond C_{10a}-N_1-C_2=O in the flavin ring I has been decreased to a great extent, on the other hand, the ring conjugation at the bond C_{4a}-C_4=O has been preserved with a little perturbation. We tentatively assign these two carbonyl stretching bands to be amide I-like modes of the ring III. These carbonyl stretching vibrational bands were not observed in the resonance Raman spectrum of lumichrome adsorbed on the metal surface as Table 1 indicates.

The ring III of lumichrome may be regarded to contain two kinds of *N*-monosubstituted secondary amide fragments in the form of *cis* configuration(CONH) if we regard two C=O stretching modes discussed above as two distinct types of amide I bands. The amide II and III bands for the *N*-monosubstituted secondary amide are representative for the *trans* configuration.[8] It is well known that no amide II and

III bands[9] are prominently distinguished for the *cis* configured secondary amides because there is no coupling between the C-N stretching vibration (usually 1350-1310 cm⁻¹) and the in-plane N-H bending vibration (usually 1490-1440 cm⁻¹).[8] The ring III of lumichrome contains two different kinds of C-N stretching modes because N_3-C_4=O or N_1-C_2=O has distinct degrees of resonance characteristics each other. The C_4-N_3 bond is more strongly resonance stiffened than the C_2-N_1 bond to come close in the range of in-plane N-H bending frequency, then to couple or interact together. Raman spectrum of lumichrome in solid phase has a band at 1279 cm⁻¹ that can be assigned due mainly to contribution from an amide III-like mode of the C_4-N_3-H vibration.[6(b)] This band gains its intensity strongly as compared to that of the FT Raman spectrum of lumiflavin, and also was not observed in the SERS of lumichrome on the silver metal surface as Table 1 indicates. Other major bands except amide I-like and amide III-like vibrations are tentatively assigned according to normal coordinate calculation studies of lumiflavin. One may indicate that the contributions to each band would be altered from those for lumiflavin, so it leaves a rigorous normal coordinate calculation study for better assignments and understandings.

Here we can enumerate two points. One is that resonance Raman spectrum of lumichrome on the colloidal silver surface listed in Table 1 did not exhibit such amide I-like or amide III-like bands. The other point is that the whole spectral feature is quite similar to resonance Raman spectra of the isoalloxazine ring, *e.g.*, lumiflavin or riboflavin. It is well understood that a metal surface charge seems to play an important role in stabilizing the adsorption and often adjusts resonance characteristics of adsorbed chromophores *via* a charge transfer mechanism or any other electrostatic phenomena. Therefore we may suggest that the ring III, particularly C_4=O, C_2=O, and N_3^-, is responsible for the adsorption of lumichrome on the metal surface as proved for lumiflavin and riboflavin previously.[1] These spectroscopic evidences support further that, upon adsorption, lumichrome becomes an isoalloxazine-like ring system indicated in Figure 1(b) with the proton transfer of N_1-H to N_{10} position. The hydrogen bonding of flavins[10] and its effect upon resonance Raman spectrum had been investigated on sites of N_1, C_2=O, N_3-H, C_4=O, and N_5. The acidity of a proton at N_3-H had been known to be higher than that of a proton at N_1-H in the isoalloxazine. The previous studies[1] and this study, therefore, confirm that its strong acidity of N_3-proton upon adsorption stems from a delocalization of the N_3 deprotonated ring III system as adsorbed on the metal surface.

Figure 1 demonstrates our spectral evidences briefly that the deprotonation at N_3-H upon adsorption on the metal surface is readily followed by the concurrent depronation at N_1 position and protonation at N_{10} position to rearrange the whole electronic structure of lumichrome, Figure 1(a), to yield an isoalloxazine-like species, Figure 1(b), which is a surface-active species.

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An Evaluation of a Polymeric Langmuir-Blodgett Film on Porous Substrate by Gas Permeability and SEM Micrographs

Jong Hyun Son, Hyein Jeong, and Burm-Jong Lee*

Department of Chemistry, Inje University, Kimhae 621-749

The Langmuir-Blodgett(LB) technique provides a unique approach to achieve supramolecular architectures of layered assemblies of suitably designed organic molecules. The LB films have been used as sophisticated molecular devices useful to test molecularly controlled processes of energy transfer, charge carrier motion, energy conversion, molecular recognition, and separation.[1] In particular, the fact that its structure is highly organized in three dimensions makes such material well-suited for adjustment and optimization of its transport properties. But, the LB films have problems to be solved for their practical applications, which are in part due to the inherent instability and the defects of the layered assemblies.[2] Specifically, when we consider its use for separations, the mechanical stability of the film should be importantly taken into account, because the separation must happen through the films that are built up on stable, porous supports. As a breakthrough to improve the mechanical sta-
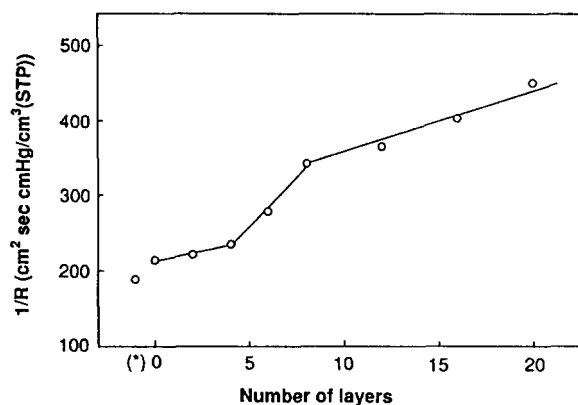

Figure 1. N_2 gas permeation rate(R) through LB films of 1 with 2 on a poly(propylene) membrane filter(METRICEL) measured by the pressure method. (*); measured without poly(propylene) substrate.

bility, the use of preformed polymers has been investigated extensively in the recent years.[3,4] Among the reports on polymeric LB films, Kunitake *et al.* have demonstrated stabilization of the LB films by electrostatic interaction of ionic polymers with oppositely-charged amphiphiles[5] and by covalent cross-linking of ionically interacting polymers.[6]

The gas transport through preformed polymeric LB films has been investigated by a few groups.[7-12] Stroeve *et al.* reported that the gas permeabilities of nitrogen, methane, and carbon dioxide in polypropylene-based asymmetric membranes were merely a function of the molecular weight of the gas.[8] Kunitake *et al.* determined the ratio α of the oxygen and nitrogen permeation rates through a LB film of an amphiphilic polymer.[9] The α value was found to be 3.2. Another study also showed that the composite films of a poly(N-dodecyl-acrylamide) revealed a higher permeation rate for oxygen.[10] In those studies of gas transport through the LB films, besides the structure of polymers employed, it is crucial for the utility of such a composite membrane that the pores of the support are homogeneously coated with the multilayer film. However, few systematic investigations on the relation between gas permeability and LB film quality have been reported.[7] Here we report a preliminary result as to the dependency of N_2 gas permeability on the macroscopic states of a polymer LB film observed by SEM.

Results and Discussion

The properties of the monolayers at the air-water interface and the resulting LB films of 1 polyion-complexed with 2 were already described.[6] The polymer 1 was spread on aqueous polyallylamine 2. The monolayer gives expanded
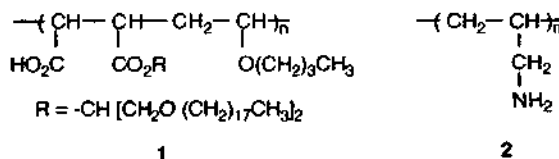
phase. The formation of a polyion complex at the interface and in the LB films could be confirmed by π-A isotherms,