not observe any apparent differences in static absorption and emission spectra between Eu^{3+} binding sC and Ca^{2+} one.

In summary, we have exchanged the Ca^{2+} ion in the weak binding site of sC for Eu^{3+} without changing the structure and function of the enzyme noticeably. The weak binding site is considerably less polar than water and has a significantly lower symmetry than an octahedral group. However, the exchanged Eu^{3+} ion of the weak binding site has four coordinated water molecules in aqueous sC solutions. The additionally coordinating room of the metal cation in aqueous sC solutions, compared with coordination in crystal structures, is suggested to play an important role for the metal cation of the weak binding site to bear a proper conformation of intermediate sC-substrate complex during enzyme reaction.

Acknowledgment. This work was supported by the Korea Science and Engineering Foundation (961-0305-038-2). We also thank the Korea Ministry of Education for partial financial support.

References

*Also a member of the Center for Molecular Science, Taejon 305-701, Korea.

1. Pantoliano, M. W.; Whitlow, M.; Wood, J. F.; Rollence, M. L.; Finzel, B. C.; Gilliland, G. L.; Poulos, T. L.; Bryan, P. N. *Biochemistry* **1988**, *27*, 8311.
2. McPhalen, C. A.; James, M. N. *G. Biochemistry* **1988**, *27*, 6582.
3. Neidhart, D. J.; Petsko, G. A. *Protein Engineering* **1988**, *2*, 271.
4. Siezen, R. J.; de Vos, W. M.; Leunissen, J. A. M.; Dijkstra, B. M. *Protein Engineering* **1991**, *4*, 719.
5. Chatterjee, S.; Russell, A. J. *Enzyme Micro. Technol.* **1993**, *15*, 1022.
6. Philipp, M.; Tsai, I.; Bender, M. L. *Biochemistry* **1979**, *18*, 3769.
7. Schmitke, J. L.; Stern, L. J.; Klibanov, A. M. *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 4250.
8. Chaudhary, A. K.; Kamat, S. V.; Beckman, E. J.; Nurok, D.; Kleyle, R. M.; Hajdu, P.; Russell, A. J. *J. Am. Chem. Soc.* **1996**, *118*, 12891.
9. Broos, J.; Visser, A. J. W. G.; Engbersen, J. F. J.; Verboom, W.; van Hoek, A.; Reinhoudt, D. N. *J. Am. Chem. Soc.* **1995**, *117*, 12657.
10. Fitzpatrick, P. A.; Ringe, D.; Klibanov, A. M. *Biochem. Biophys. Res. Commun.* **1994**, *198*, 675.
11. Bode, W.; Papamokos, E.; Musil, D. *Eur. J. Biochem.* **1987**, *166*, 673.
12. Kim, D. U.; Choi, M.-U. *Bull. Korean Chem. Soc.* **1989**, *10*, 600.
13. Kidd, R. D.; Yennawar, H. P.; Sears, P.; Wong, C.-H.; Farber, G. K. *J. Am. Chem. Soc.* **1996**, *118*, 1645.
14. Horrocks, Jr., W. D.; Sudnick, D. R. *Acc. Chem. Res.* **1981**, *14*, 384.
15. Bunzli, J.-C. G.; Choppin, G. R. *Lanthanide Probes in Life, Chemical and Earth Sciences*; Elsevier Science: Amsterdam, The Netherlands, 1989; p 1.
16. Snyder, A. P.; Sudnick, D. R.; Arkle, V. K.; Horrocks, Jr., W. D. *Biochemistry* **1981**, *20*, 3334.
17. Oomen, E. W. J. L.; van Dongen, A. M. A. *J. Non-Cryst. Sol.* **1989**, *111*, 205.

Resonance Raman Scattering and Surface-Enhanced Resonance Raman Scattering of Ru(II) Complexes

Young Mee Jung, Sang Il Nam, Jong Wan Lim, Hwan Jin Yeo, and Mu Sang Lee*

Department of Chemistry Education, Kyungpook National University, Taegu 702-701, Korea
Received October 23, 1997

It has been recognized that surface-enhanced Raman scattering (SERS) is a very useful technique for studying the adsorption process of molecules on metal surface.[1-5] When a molecule is adsorbed on metal surface, its Raman intensities can be enhanced by five to six orders of magnitude so that the normally insensitive Raman technique is used to study adsorbed species at micromolar concentrations. For molecules which absorb light at an appropriate laser wavelength, the combination of resonance enhancement and surface enhancement (surface-enhanced resonance Raman scattering) can lead to as much as twelve orders of magnitude enhancement.[6]

Inorganic complexes have many advantages over the more studied organic molecules due to their higher symmetries, various charges, and various physicochemical properties such as hydrophobic/hydrophilic nature. In this sense, ruthenium(II) complexes containing π-conjugated ligands have become the focus of a variety of photochemical, electrochemical and spectroscopic investigations.[7-9] Resonance Raman spectra and surface-enhanced resonance Raman spectra of $[Ru(bpy)_3]^{2+}$ have also been reported.[6] Their wavelength excitation profiles in SERRS are supposed, however, to alter substantially due to the surface selection rules involved.[5,10-13]

In this paper, we have used SERS to prove the interactions of $[Ru(bpy)_2L]^{2+}$ complexes with the silver colloidal surface. Because of the luminescence of some of these compounds, this study was performed with SERRS.

Experimental Section

Aqueous silver colloid was prepared according to the method of Creighton *et al.*[14] Prepared silver sols were yellow, displaying a single absorption maximum at 395.4 nm.

Distilled water used in this work was obtained by using Barnsted water purification system. $RuCl_{33}H_2O$, 2,2'-Bipyridine (bpy), 1,10-phenanthroline (phen), 4,7-Diphenyl-1,10-phenanthroline (bathophen), 4-nitro-1,10-phenanthroline (NO_2 phen), 3,4,7,8-tetramethyl-1,10-phenanthroline (Me_4-phen) and 4,7-dimethyl-1,10-phenanthroline (Me_2phen) were obtained from Aldrich Chemical Co. and used as received. The complexes $[Ru(bpy)_2L]^{2+}$ were prepared following the literature procedures.[15] The solutions of Ru(II) complexes were prepared following dissolving small amounts of these solids in acetonitrile.

The SERRS spectra were obtained using a Raman spectrometer equipped with a SPEX 1403 scanning double monochromator, an RCA C31034 PMT detector, and SPEX DM 3000R softwares. Coherent Innova 90-5 argon ion laser (λ= 514.5, 488.0 and 476.5 nm) was used as the light source. All the spectra shown were obtained at a scan rate of 0.2 cm^{-1}/sec with a slit width of 400/400/400/400 μm. UV-vis spectra were obtained in quartz cells by using a Shimadzu UV-2101 PC instrument.

Results and Discussion

It is impossible to record the Raman spectra of $[Ru(bpy)_2L]^{2+}$ complexes with 514.5 nm laser excitation owing to the strong luminescence of the compound. But with 476.5 nm laser exitation Raman peaks were clearly enhanced. This can be understood by recalling that for $[Ru(bpy)_2L]^{2+}$ complexes, the metal-to-ligand charge transfer (MLCT) band is observed at 450 nm region in their absorption spectra. Namely, their resonance Raman (RR) spectra can be obtained by an excitation within in MLCT (d-π*) absorption band.

Figure 1 shows the resonance Raman spectra of $[Ru(bpy)_2L]^{2+}$ complexes with 476.5 nm laser excitation. It is clear from Figure 1 that vibrational bands in the 1300-1500 cm^{-1} region are stronger than those in other regions. This may reflect that the C = C and C = N bonds are affected mostly by the electron transfer from metal d-orbitals to ligand pi system. Since the resonance Raman spectra of $[Ru(bpy)_2L]^{2+}$ complexes obtained in this study are quite similar to that of $[Ru(bpy)_3]^{2+}$ reported in the literature,[6] many ring modes are supposed to be actively involved in the CT transition.

Figure 2 shows the SERS and SERRS spectra of 10^{-3} M $[Ru(bpy)_2(Me_4phen)]^{2+}$, which is one of $[Ru(bpy)_2L]^{2+}$ complexes studied in this work, taken with 476.5, 488.0 and 514.5 nm laser excitation. In Figure 2, we can see the surface enhancement by comparing with the Raman spectrum shown in Figure 1. As the laser excitation frequency is increased from 514.5 (Figure 2c) to 476.5 nm (Figure 2a), the peak intensity is remarkably increased in the SERRS spectrum.

Five SERRS spectra of 10^{-3} M $[Ru(bpy)_2L]^{2+}$ taken with 476.5 nm excitation are shown in Figure 3. In Figure 3b, the predominant band around 1440 cm^{-1} may be assigned to the coupled vibration of aromatic rings of bathophen ligand. As shown in the RR and SERRS spectra of $[Ru(bpy)_2L]^{2+}$, adsorption of the metal complexes on the partially coagulated silver colloids permits the observation of SERRS spectra when the excitation wavelength is close to the absorption maximum of the MLCT band and the surface-

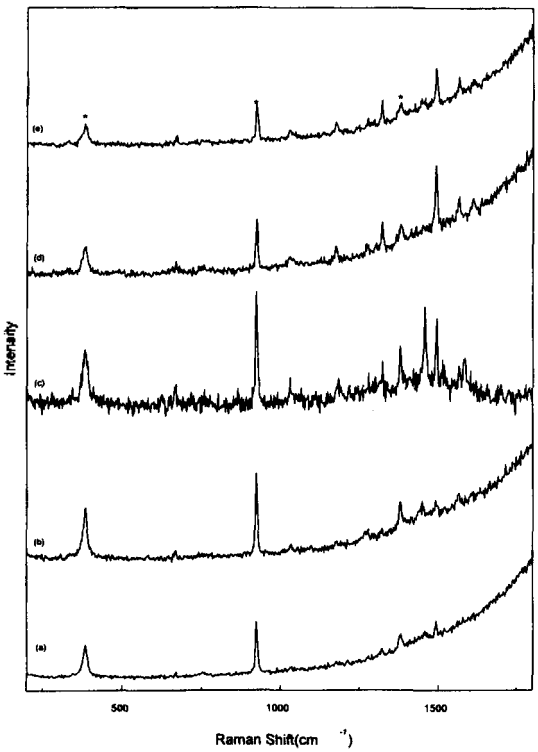

Figure 1. Raman spectra of $(1 \times 10^{-3}$ M) $[Ru(bpy)_2L]^{2+}$ at 476.5 nm. (a) $[Ru(bpy)_2(phen)]^{2+}$, (b) $[Ru(bpy)_2(bathophen)]^{2+}$, (c) $[Ru(bpy)_2(NO_2phen)]^{2+}$, (d) $[Ru(bpy)_2(Me_4phen)]^{2+}$, (e) $[Ru(bpy)_2(Me_2phen)]^{2+}$, * solvent peak.

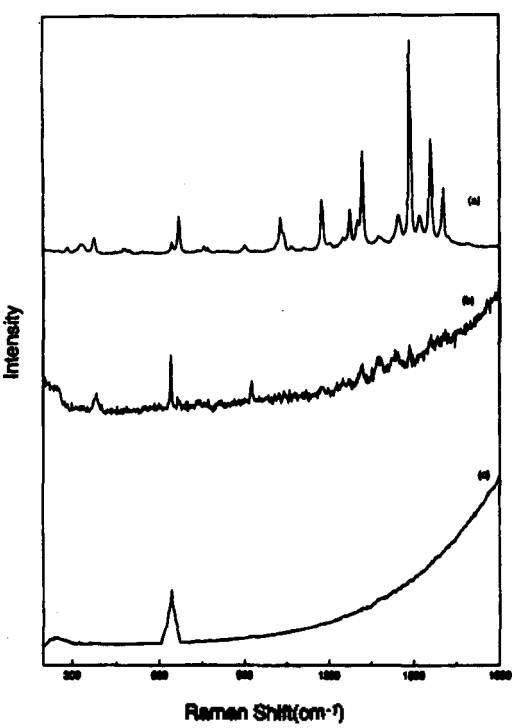

Figure 2. SERS and SERRS spectra of $(1 \times 10^{-3}$ M) $[Ru(bpy)_2(Me_4phen)]^{2+}$ in (a) 476.5 nm, (b) 488.0 nm and (c) 514.5 nm.

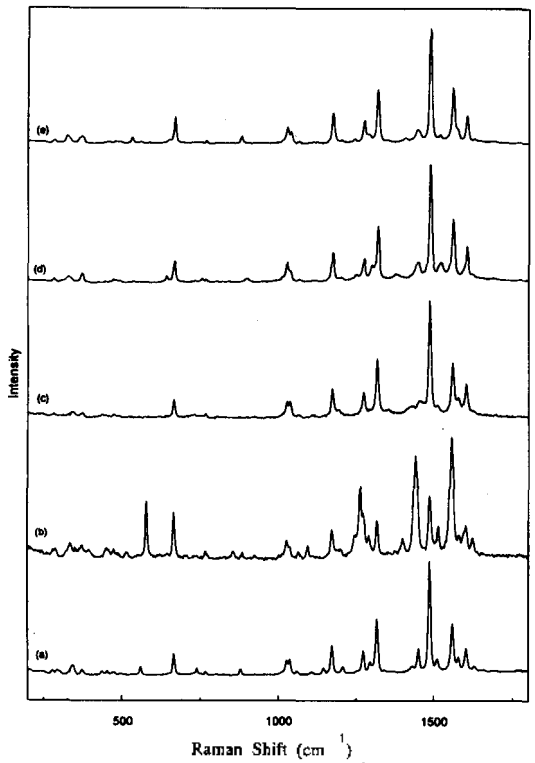

Figure 3. SERRS spectra of $[Ru(bpy)_2L]^{2+}$ at 476.5 nm. (a) $[Ru(bpy)_2(phen)]^{2+}$, (b) $[Ru(bpy)_2(bathophen)]^{2+}$, (c) $[Ru(bpy)_2(NO_2phen)]^{2+}$, (d) $[Ru(bpy)_2(Me_4phen)]^{2+}$, (e) $[Ru(bpy)2(Me_2phen)]^{2+}$.

plasmon wavelength. There is an apparent surface enhancement of *ca* 10^6, caused by the adsorption of complex onto the silver particles in addition to the resonance enhancement. When Ru(II) complex ions are adsorbed onto the colloid, the intense luminescence observable at a 514.5 nm excitation is apparently quenched at a 476.5 nm excitation. The vibrational assignments of five complexes are made by referring to those of $[Ru(bpy)_3]^{2+}$ complex reported in the literature, and are given in Table 1. The vibrations of $[Ru(bpy)_2L]^{2+}$ cations can be classified into three groups; ring modes, inter-ring vibrations (within a bipyridine ligand), and metal-ligand modes. The SERRS spectra of $[Ru(bpy)_2L]^{2+}$ are similar to the RR spectra, despite of poor quality due to the luminescence, they show only very small wavenumber shift. This means that molecules may be physisorbed weakly and do not interact chemically with the silver colloid. The SERRS spectrum of $[Ru(bpy)_2L]^{2+}$ was similar to the spectrum of $[Ru(bpy)_3]^{2+}$ adsorbed on the silver colloid[16] and the RRS spectrum of $Ru(bpy)_3Cl_2$.[17] This suggests that the nitrogen atoms of the ligand are similarly coordinated to those in $[Ru(bpy)_3]^{2+}$ and $Ru(bpy)_2Cl_2$. As shown in Figure 3, the SERRS spectra of Ru(II) complexes are similar to one another except for $[Ru(bpy)_2(bathophen)]^{2+}$. It is clear from Figure 3b that the spectrum shows mainly the contributions from the bathophen ligand with a little contribution from the bpy ligand. Recalling the literature[6] data in $[Ru(bpy)_3]^{2+}$, we can thus suggest that the electron is transferred from metal (Ru) to the ligand such that

$$[Ru(bpy)_2(phen)]^{2+} \xrightarrow{476.5\ nm} [Ru^{III}(bpy)_2^-(phen)]^{2+}$$

$$[Ru(bpy)_2(bathophen)]^{2+} \xrightarrow{476.5\ nm} [Ru^{III}(bpy)_2(bathophen)^-]^{2+}$$

$$[Ru(bpy)_2(NO_2phen)]^{2+} \xrightarrow{476.5\ nm} [Ru^{III}(bpy)_2^-(NO_2phen)]^{2+}$$

$$[Ru(bpy)_2(Me_4phen)]^{2+} \xrightarrow{476.5\ nm} [Ru^{III}(bpy)_2^-(Me_4phen)]^{2+}$$

$$[Ru(bpy)_2(Me_2phen)]^{2+} \xrightarrow{476.5\ nm} [Ru^{III}(bpy)_2^-(Me_2phen)]^{2+}$$

Table 1. Details of surface-enhanced Raman spectra of $[Ru(bpy)_2L]^{2+}$ complexes

L= phen	batho-phen	NO_2 phen	Me_4 phen	Me_2 phen	Assignment
347	339	-	-	327	ν Ru-N
-	-	-	378	378	
-	456	-	-	-	Inter-ring
668	668	663	671	671	δ(CCC) inter-ring
883	-	-	-	883	δ(CCH)oop
1029	1029	1032	1029	1029	ring breathing
1041	-	-	-	1040	
1177	1174	1177	1174	1177	δ(CCH)ip
1276	1267	1275	1275	1279	δ(CH)+ν(ring)
1318	1318	1322	1318	1318	ν(C-C) inter-ring
-	1401	-	-	-	
1452	1440	1456	1448	1448	ν C-N
1491	1491	1491	1491	1491	δ CHip
-	-	-	1522	-	ν C=C
1561	1557	1561	1561	1561	
1604	1604	1604	1604	1608	

We have obtained the SERRS spectra of $[Ru(bpy)_2L]^{2+}$ in various concentrations. As the concentration of Ru(II) complex is decreased, the peak position is hardly to change while the peak intensity is diminished. The minimum concentration at which SERRS may be obtained is 10^{-12} molL^{-1}. When lower concentrations of the complexes were employed ($\leq 10^{-6}$ molL^{-1}), SERRS spectra could be obtained only by the addition of an electrolyte solution. The addition of a 1 molL^{-1} KCl solution enabled SERRS spectra of complexes to be measured at concentration as low as 10^{-12} molL^{-1}.

Conclusion

Absorption of the metal complexes on partially coagulated silver sol colloids permits the observation of SERRS spectra when the excitation wavelength is close to the absorption maximun of the MLCT band and the surface-plasmon wavelength. In the SERRS of Ru(II) complexes, the vibrations of C ··· C and C ··· N are stronger than other bands. These results suggest that the bands of C ··· C and C ··· N are affected greatly by the transfer of electron density from metal d-orbitals into ligand pi system.

The fluorescence background is reduced owing to the interaction between the adsorbate and the surface as shown in SERS spectra of $[Ru(bpy)_2L]^{2+}$ complexes. The electromagnetic field is responsible for the majority of the enhancement in SERS spectra of these complexes. A detection limit of 10^{-12} molL^{-1} for $[Ru(bpy)_2L]^{2+}$ complexes in SERRS can be achieved by a combination of surface enhancement and resonance enhancement.

Acknowledgement. The financial support in part by the Atomic Energy Research Fund, Ministry of Science and Technology, by the 95 special Fund for University Research Institute, Korea Research Fund are greatly appreciated.

References

1. Chang, R. K.; Furtak, T. E. Eds.. *Surface enhanced Raman scattering*; Plenum: New York, 1982.
2. Fleischman, M.; Hendra, P. J.; Mcquillaan, A. J. *Chem. Phys. Lett.* **1974**, *26*, 163.
3. Jeanmaire, D. L.; Van Duyne, R. P. *J. Electronal. Chem.* **1977**, *1*, 84.
4. Albrecht, M. G.; Creighton, J. A. *J. Am. Chem. Soc.* **1977**, *99*, 5215.
5. Moskovits, M. *Rev. Mod.* **1985**, *57*, 783.
6. Dines, T. J.; Peacock, R. D. *J. Chem. Soc., Faraday Trans.* **1989**, *1*(84), 3445.
7. Calvert, J. M.; Meyer, T. J. *Inorg Chem.* **1982**, *20*. 27.
8. Rubenstein, I.; Buck, R. P. *J. Electronal. Chem.* **1982**, *140*, 173.
9. Hari, Virdes, R. *J. Phys. Chem.* **1984**, *88*, 451.
10. Moskovits, M. *J. Chem. Phys.* **1982**, *77*, 4408.
11. Cotton, T. M.; Uphaus, R. A.; Mobius, D. *J. Phys. Chem.* **1986**, *90*, 6071.
12. Moskovits, M.; Suh, J. S. *J. Phys. Chem.* **1984**, *88*, 1293.
13. Moskovits, M.; Suh. J. S. *J. Phys. Chem.* **1984**, *88*, 5526.
14. Creighton, J. A.; Blatchford, C. G.; Albercht, M. J. *J. Chem. Soc., Faraday Trans* **1979**, *75*, 790.
15. Takeuchi, K. J.; Thompsons, M. S.; Pipes, D. W.; Meyer, T. J. *Inorg. Chem.* **1984**, *23*, 1845.
16. Vanhecke, F.; Heremans, K. *J. Raman Spectrosc.* **1989**, *20*, 617.
17. Basu, A.; Gafney, H. D.; Strekas, T. C. *Inorg. Chem.* **1982**, *21*, 2231.

Multiple Activities of Oleate-Activated Phospholipase D Exist in Rat Brain Microsomes Solubilized by Decanoyl *N*-Methylglucamide

Bong-sub Kim, Seongtaek Hong, and Myung-Un Choi*

Department of Chemistry and Center for Molecular Catalysis, Seoul National University, Seoul 151-742, Korea
Received October 28, 1997

Phospholipase D (PLD) has emerged as an enzyme involved in signal transduction, vesicle trafficking, and membrane remodeling.[1] The enzyme was discovered in plants 50 years ago, but distribution of PLD has been found to be extremely widespread in the most of biological sources.[1,2] The PLD activity in mammalian tissue was initially detected in 1973 by Saito and Kanfer[3] and activation of PLD by oleate was reported in 1982 by the same group.[4] Since then the PLD assay system containing oleate has been tested and the oleate-activated PLD was found in a variety of mammalian tissues.[5-8] Further evidence obtained in a variety of biochemical studies implicate the existence of multiple isoforms of PLDs.[9] Two isoforms of PLD identified in mammalian sources indicate that PLD1 localized in the perinuclear region is dependent on ADP-ribosylation factor (ARF) and phosphatidylinositol 4,5-bisphosphate (PIP_2), whereas PLD2 in plasma membrane is regulated by PIP_2 but not by ARF.[10] In addition to membrane-associated PLDs, a cytosolic PLD activity was identified in human neutrophils and bovine lung.[11] Therefore resolution of multiple isoforms and search for their physiological roles have been major focusing effort in signal-activated PLDs. Recent advances in the PLD study have brought new insights into the molecular and cellular roles of PLD. PLDs have been cloned from caster bean, rice, maize, Arabidopsis, yeast, human, and mouse.[12] Analysis of the cDNA sequence of PLD has led to the identification of probable catalytic and regulatory domains.[13]

In spite of many attempts to solubilize membrane-associated PLD, no animal PLDs have been fully purified and characterized.[14-19] In this work, the oleate-activated PLD of microsomal membrane from rat brain was solubilized using decanoyl N-methylglucamide (MEGA-10). The stability of solubilized PLD improved substantially from previous study[19] and the source was further fractionated into 4 fractions by chromatography on Sephacryl S-300. Here we report a partial characterization of multiple PLD activities in brain microsomes, which are activated by oleate.

Experimental

Radioactive 1,2-di[1-^{14}C]palmitoyl-L-3-phosphatidylcholine (specific activity 111 mCi/mmole) was purchased from Amersham (Aylesbury, England). Phosphatidic acid and phosphatidylethanol were prepared from phosphatidylcholine using cabbage PLD according to the procedure described previously.[20] Sodium oleate, decanoyl N-methylglucamide, and Sephacryl S-300 were obtained from Sigma (St. Louis, USA). Precoated silica gel 60 TLC plate was purchased from Merck (Darmstadt, Germany). Brain microsomal fraction was obtained from female Wistar rats (four weeks old). The brains were homogenized in ten volume of 10% sucrose solution and centrifuged at 10,000×g for 10 minutes at 4°C. The supernatant was recentrifuged at 100,000×g for 1 hour to obtain microsomal pellet. Initially the microsomal pellet was