Surface-Enhanced Raman Spectroscopy from Flavins Adsorbed on a Silver Electrode: Observation of the Unstable Semiquinone Intermediate

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Abstract: The surface-enhanced Raman scattering (SERS) spectroscopy of protein-free flavin in different redox states was investigated at a silver electrode. Good-quality spectra for oxidized flavin with an excitation frequency within the absorption band (514.5 or 488 nm) and out of the absorption band (yellow-red region) are reported. Fluorescence interference from the flavin is nearly completely quenched by the surface interaction. Reduced flavin did not exhibit a well-defined SERS spectrum, probably because of the break down of the surface complex. The SERS spectrum of the neutral semiquinone radical, as an intermediate of the two single-electron reduction steps, was observed in acidic solution with yellow or red excitation. The utility of SERS as a technique for probing the existence of an unstable intermediate species at the electrode surface is demonstrated.

The use of surface-enhanced Raman scattering (SERS) spectroscopy for the study of biologically important molecules and other complex molecules is now well established.[1] The major advantages of SERS are its sensitivity which allows its use as an in situ method for examining molecules at interfaces and the quenching of fluorescence background which is often associated with complex molecules. The latter advantage is particularly important for the flavin molecules which fluoresce so strongly that resonance Raman (RR) spectra from visible laser excitation cannot be observed in solution without adding protein or KI as a quencher. The aforementioned attributes of SERS are utilized in this paper to study the surface Raman spectroscopy of flavin systems under electrode potential control.

A considerable number of resonance Raman studies have been made of flavins and flavoproteins with various methods[2-12] to conquer the difficulty of fluorescence interference. The most recent methods used for avoiding fluorescence interference in flavin resonance Raman investigations have been ultraviolet RR spectroscopy[8] and SERS studies utilizing both Ag colloids[9-11] and Ag electrodes.[12] High-quality spectra from flavoproteins[8] and free flavins[9] were reported on silver colloidal particles upon the combination of fluorescence quenching and Raman intensity enhancement provided by the silver surface. However, evidence has now been presented which shows that the spectra from the flavoprotein originates from free flavin extracted from the protein on both Ag colloids[11] and electrodes.[12] It appears that there is little surface enhancement for the protein-bound flavins where the flavin cannot chemisorb to the surface. On the other hand, the free flavins give intense SERS spectra at very low concentrations,[11,12] indicating strong surface interaction and a chemical component to the enhancement.[12] In order to further explore the possibility of applying SERS for the study of flavin systems, we have carried out SERS measurements at silver electrodes which allow one to monitor flavin reduction reactions and obtain in situ spectral information at the same time.

The flavin molecule is an important redox coenzyme which can be involved in single-step two-electron processes for organic oxidation or reduction reactions or in two-step one-electron processes for electron transfer to or from metal centers. The ability of flavins to operate in either mode is practically unique among biomolecules.[13-15] It is known that the tendency of the flavin toward one- or two-electron oxidation or reduction processes and the electrochemical potential of flavins are substantially affected by pH, by the polarity of the environment, and by interaction with metal ions. A study of these effects should reveal much about the methods open to proteins in controlling the reactivity of these coenzymes.

We have examined the electron-transfer process of flavin reduction in different pH solutions with voltammetry and in situ SERS spectroscopy. A spectrum, which was obtained from an acidic solution at the flavin reduction potential, was identified as being due to the semiquinone radical of the flavin. The semiquinone radical is formed by a single electron transfer and is an intermediate in the overall two-electron reduction. Cyclic voltammetry shows that the potential separation between the two single electron transfer steps changes with varying pH and the maximum value is about 50 mV. In such a case, electrochemical measurement cannot determine whether a twofold uptake of single electrons via an unstable intermediate or a single two-electron reduction process occurs.[16] To our knowledge this is the first time that SERS spectroscopy has proved to be an effective approach for detecting unstable electrochemical intermediates at an electrode surface.

Experimental Section

Riboflavin (RF), flavin mononucleotide (FMN), flavin adenine dinucleotide (FAD), and D_2O were purchased from Sigma Chemical Co. and used as received. Reagent grade KNO_3, K_2SO_4, KCl, and KBr served as supporting electrolytes. The solutions were prepared with deionized distilled water on the same day of the experiment and deaerated by nitrogen bubbling for 20 min. The pH of the solution was adjusted by adding reagent grade HNO_3 and NaOH and measured by an Orion Research digital ionalyzer Model 801 A.

(1) Cotton, T. M. In *Surface and Interfacial Aspects of Biomedical Polymers*; Anderade, J. B., Ed.; Plenum: New York, 1985; Vol. II, p 161.
(2) Dutta, P. K.; Nestor, J. R.; Spiro, T. G. *Proc. Natl. Acad. Sci. U.S.A.* **1977**, *76*, 4146-4149.
(3) Nishina, Y.; Kitagawa, T.; Shiga, K.; Horiike, K.; Matsumura, Y.; Watari, H.; Yamano, T. J. *Biochemistry (Tokyo)* **1978**, *84*, 925-932.
(4) Schmidt, J.; Coudron, P.; Thompson, A. W. Watters, K. L.; McFarland, J. T. *Biochemistry* **1983**, *22*, 76-84.
(5) Kitagawa, T.; Nishina, Y.; Kyogoku, Y.; Yamano, T.; Ohishi, N.; Takai-suzuki, A.; Yagi, K. *Biochemistry* **1979**, *18* (9), 1804-1808.
(6) Schopfer, L.; Morris, M. *Biochemistry* **1980**, *19*, 4932.
(7) Schopfer, L.; Haushlter, J.; Smith, M.; Miland, M.; Morris, M. *Biochemistry* **1981**, *20*, 6734-6739.
(8) Copeland, R. A.; Spiro, T. G. *J. Phys. Chem.* **1986**, *90*, 6648-6654.
(9) Copeland, R. A.; Fodor, S. P. A.; Spiro, T. G. *J. Am. Chem. Soc.* **1984**, *106*, 3872-3874.
(10) Lee, N. S.; Sheng, R. S.; Morris, M. D.; Schopfer, L. M. *J. Am. Chem. Soc.* **1986**, *108*, 6179-6183.
(11) Lee, N. S.; Hsieh, Y. Z.; Morris, M. D.; Schopfer, L. M. *J. Am. Chem. Soc.* **1987**, *109*, 1358-1363.
(12) Holt, R. E.; Cotton, T. M. *J. Am. Chem. Soc.* **1987**, *109*, 1841-1845.
(13) Hemmerich, P. In *Bioinorganic Chemistry II*; Raymond, K. N., Ed.; American Chemical Society: Washington, DC, 1977; p 312.
(14) Hemmerich, P.; Massey, V. In *Oxidases and Related Redox Systems*; King, T. E., Mason, H. S., Morrison, M., Eds.; Pergamon: Oxford, 1979.
(15) Walsh, C. *Acc. Chem. Res.* **1980**, *13*, 148-155.
(16) Bard, A. J.; Faulkner, L. R. *Electrochemical Methods*; John Wiley and Sons, Inc.: New York, 1980; pp 232-236.

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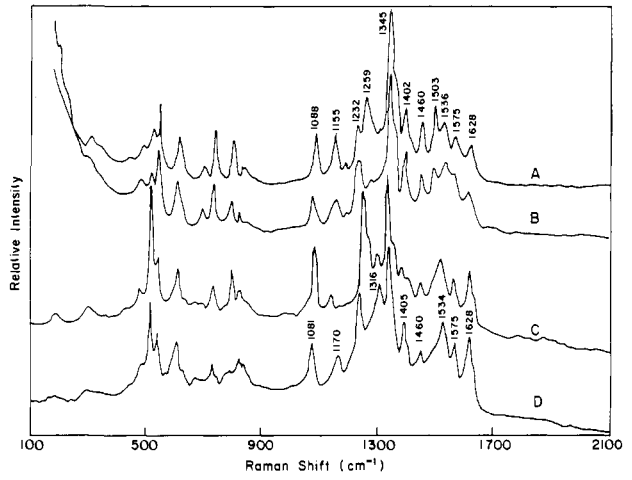

Figure 1. SERS and SERRS spectra of oxidized RF (10^{-5} M) on a Ag electrode in pH 6.8 (or pD 6.8) solution with 0.1 M K_2SO_4 as electrolyte at −0.4 V vs. SCE. Excitation frequency 585 nm in H_2O (A) and in D_2O (B) and 514.5 nm in H_2O (C) and in D_2O (D).

The SERS experimental setup was similar to that described elsewhere.[17] A Spectra Physics 164 argon ion laser at 488 and 514.5 nm was used as an excitation source. Yellow or red light was obtained with a Spectra Physics Model 375 tunable dye laser. The laser power at the electrode was approximately 40 mW. Spectra were recorded with a Spex Model 1401 double monochromater with a wavenumber resolution of around 4 cm^{-1}. Photon counting detection was used with an accumulation time of 0.6 s/cm^{-1}, and the intensities were recorded digitally and are presented unsmoothed.

The sample cell consisted of a silver working electrode with ca. 1.6 mm^2 surface area, a Pt counter electrode, and a saturated calmel electrode (SCE) as the reference. All potentials in this paper are quoted vs. SCE. The oxidation-reduction-cycle (ORC) pretreatment was accomplished in the presence of flavin molecules by applying a triangular sweep, 50 mV/s, from the initial potential to a switching potential of +0.25, +0.5, and +0.6 V for KCl, K_2SO_4, and KNO_3 electrolytes, respectively. No difference in spectral features was observed from these three electrolyte solutions. Since the flavin is reduced at rather positive potentials in acidic solutions, we used K_2SO_4 in most cases in order to start electrode potential scans at 0.0 V. The ORC pretreatments were carried out in the dark and at neutral pH, and then the solution pH could be adjusted to a certain value by adding HNO_3 or NaOH solution. With yellow or red excitation the SERS spectrum could be observed when the flavin was added after an ORC pretreatment which was performed in a pure K_2SO_4 or KCl solution. In this case a flavin concentration higher than 10^{-5} M is required, while more intense spectra can be obtained from 10^{-7} M when the flavin is present during the ORC. When 488- or 514.5-nm excitation (in the absorption band) was used, it was found that a much better SERS spectrum could be obtained by eliminating the fluorescence from the flavin in the bulk solution by physically washing out the test solution with electrolyte blank after the ORC.

Results and Discussion

Surface Raman Spectra of Oxidized Flavin. Figure 1A shows the SERS spectrum of oxidized RF adsorbed at a roughened silver electrode at −0.4 V vs, SCE when excited with 585-nm laser light. The solution contains 10^{-5} M RF and 0.1 M K_2SO_4 at a pH value of 6.8 for which the electrochemical reduction of RF occurs at an electrode potential more negative than −0.50 V vs. SCE as found by cyclic voltammetry. Two other substituted flavins, FMN and FAD, exhibit the same SERS spectrum as RF, showing the side chain of the flavin is not involved in the surface enhancement. The SERS spectrum clearly reveals the same features of the flavin vibrational spectrum as the normal Raman (NR) spectrum.[18]

The spectrum of Figure 1C was obtained under the same conditions as that for Figure 1A except that the excitation frequency was changed from 585 to 514.5 nm which is within the absorption band of the flavin. A 488-nm excitation gives exactly

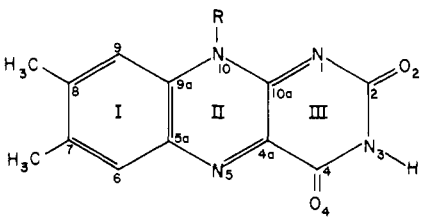

Figure 2. Numbering of the isoalloxazine ring of the flavin.

the same spectrum as Figure 1C. In order to obtain these surface-enhanced resonance Raman scattering (SERRS) spectra, the electrode was positioned within 4 mm of the cell window to reduce the fluorescence from the solution. It is possible to completely remove this fluorescence background by washing out the unadsorbed flavin molecules from the cell with pure electrolyte solution. This indicates a high fluorescence quenching efficiency of the surface interaction in contrast to quenching with a protein or KI. Furthermore, the spectrum taken after washing does not show any changes in band frequency or relative intensity with respect to the spectrum obtained before washing, indicating that there is no RR component from the solution in the observed surface spectrum.

It can be seen that the 514.5-nm spectrum (Figure 1C) is not greatly different from the 585-nm spectrum (Figure 1A). However, several bands do show different relative intensities and there are also a few bands which shift in frequency. One possible explanation is that the Raman scattering processes are different and another possibility is that the RF undergoes a photochemical reaction to lumiflavin (the side chain of RF is broken and a CH_3 group is added) under 514.5-nm laser exposure (40 mW, 20 min). Lee et al.[10] found that with prolonged laser exposure (488 nm, 20 mW, 1 h) on a silver sol system, a lumiflavin SERRS spectrum can be observed with a RF sample. In order to investigate whether there was photochemistry in our experiment, we first took a spectrum with 585-nm laser excitation followed directly with a second spectrum where the only change was the utilization of 514.5-nm excitation; and again we obtained a third spectrum with 585-nm excitation under the same conditions. Each spectrum took ca. 20 min so that the three scans were completed in an hour. The first and third spectra show exact agreement in both band frequencies and relative intensities and are identical with Figure 1A. The second spectrum is different and appears as Figure 1C. These observations imply that the difference between the two spectra is due to a resonance effect rather than a photochemical reaction. Such effects from differences in the Raman scattering process with different excitation frequencies have recently been discussed in the literature.[8] In the present work, this explanation for the observed differences is supported by the parallel behavior of NR and RR spectra of the flavins (Table I). Bands VI and XIII shift down by ca. 7 cm^{-1} when the excitation is changed from the red into the resonance frequency in both solution and surface spectra. This can be explained by a different resonance enhancement effect on each vibrational mode. When an observed band is a composite of several overlapped vibrational modes, which is the case in the flavin spectrum, a difference of 7 cm^{-1} in the two spectra is possible. Furthermore, the SERS and NR[18] spectra show similar features in their relative intensity pattern while the SERRS and RR[5] spectra are similar to each other despite some frequency shifts of the surface spectra from the solution spectra.

Spectra B and D in Figure 1 were obtained from D_2O solution where the N_3–H is deuteriated in the SERS and SERRS spectra. As in the case of solution Raman studies the change occurs in the 1300–1100-cm^{-1} region (Table I). In the solution Raman spectra, band X shifts about 40 cm^{-1} to higher frequency, which has been interpreted as the result of a decrease in the N_3–H (see Figure 2 for the numbering of the atoms of the isoalloxazine ring) bending character after deuteriation leading to an up-shift toward the intrinsic frequency of the C=O vibrations.[19] A recent normal

(17) Birke, R. L.; Lombardi, J. R.; Sanchez, L. A. *Surface Enhanced Raman Spectroscopy*; Kadish, K. M., Ed.; American Chemical Society: Washington, DC, 1982; Adv. Chem. Ser. No. 201, Chapter 4.
(18) Nisihmura, Y.; Tsuboi, M. *Chem. Phys. Lett.* **1978**, *59* (*2*), 210–213.
(19) Dutta, P. K.; Nestor, J.; Spiro, T. G. *Biophys. Res. Commun.* **1978**, *83* (*1*), 209–216.

Table I. Flavin N_3-H(D) Isotopic Frequency Shifts[e]

band label	riboflavin on Ag electrode				FMN		RF(protein)		RF(Ag sol	
	SERS (585[d])		SERRS (514.5)		NR[a] (600)		RR[b] (488)		SERRS[c] (488)	
	H_2O	D_2O	H_2O	D_2O	H_2O	D_2O	H_2O	D_2O	H_2O	D_2O
I	1628 m	1628 m	1628 s	1628 s	1633 m	1628 m	1631 s	1630 s	1630 vs	1630 vs
II	1575 m	1575 m	1575 m	1575 m	1584 m	1584 m	1584 s	1582 s	1576 m	1577 m
III	1536 m	1538 m	1535 m	1534 m	1551 m	1548 m	1548 w	1548 w	1536 w	1536 m
IV	1503 m	1502 m	1502 m	1500 sh	1503 m	1502 m	1503 m	1499 m	1507 m	1507 m
V	1460 m	1458 m	1462 w	1460 w	1469 m	1468 m	1465 w	1462 w	1465 w	1465 w
			1416 sh				1420 sh	1420 sh		
VI	1402 s	1403 s	1395 m	1405 m	1413 s	1411 s	1407 s	1406 s	1409 m	1409 m
VII	1345 vs	1348 vs	1344 vs	1348 vs	1355 vs	1351 vs	1355 vs	1351 vs	1349 vs	1350 vs
VIII			1308 w				1302 w		1308 sh	1309 sh
IX			1268 sh		1282 w					
X	1259 s	1281 w	1256 vs	1316 m	1261 m	1300 w	1252 s	1295 s	1287 s	1289 s
XI	1232 m	1242 m		1244 s	1233 s	1230 m	1229 s	1232 w	1235 m	1246 m
XII	1186 w	1188 w			1187 m	1172 m	1179 w	1181 w	1190 w	1190 w
XIII	1155 m	1172 m	1147 w	1170 m	1166 m	1148 m	1161 m	1147 m	1160 m	1163 m
XIV	1088 m	1084 m	1088 s	1081 s				1138 m	1092 m	1090 m

[a] Reference 18. [b] Reference 5. [c] Reference 10. [d] Excitation frequency (nm). [e] vs, very strong; s, strong; m, medium; w, weak; sh, shoulder.

coordinate analysis[20,21] for flavins shows that band X involves a skeletal stretching vibration in ring III of the N_1, C_2, N_3, C_4 atoms which are coupled to the N_3-H bending mode. Substitution of a heavier atom for hydrogen in this bending mode lowers the coupling leading to an up-shift in the band X stretching vibration. In our surface spectra this band also disappears from the 1259-cm^{-1} region. However, the new band appears weakly at 1281 cm^{-1} in the D_2O SERS spectrum, while it seems to be overlapped at 1316 cm^{-1} with the 1303-cm^{-1} band in the D_2O SERRS spectrum. Neither of these bands can be assigned with certainty. Nevertheless, the unambiguous isotopic effect in the 1300–1100-cm^{-1} region is in sharp contrast to the spectra from H_2O and D_2O in Ag sol experiments[10] where the SERRS spectra are identical (Table I) and where the N_3-deprotonated anion was found to be the SERS active species.

In addition, the authors[10] found that making the Ag colloidal solution acidic produces gradual attenuation with an abrupt loss of signal around pH 4, while we observed the SERS and SERRS spectra of flavins at pH 2 with about equal intensity as observed in the pH 7 solution both at 0 V vs. SCE. The N_3 protonated neutral flavin is thus suggested to be the SERS active species on the Ag electrode which probably has a different surface charge than the silver colloidal particles. The difference in protonation state of the SERS active species on the electrode and sols is clearly reflected in the surface spectra where band X shifts up in Ag sols by ca. 35 cm^{-1} from its position in the solution RR spectrum, while it remains virtually unchanged on Ag electrodes (Table I). Here we see an example of the phenomenon that SERS spectra on Ag electrodes may show differences from analogous results on Ag sols. We suggest that the differences are due to different surface conditions in the two cases, depending on the experimental conditions, such as differences in surface charge. The fact that the supporting electrolyte concentration is much lower in the Ag sols than in the Ag electrode system does not seem to be the source of the observed spectral differences. Flavin SERRS and SERS spectra measured on the Ag electrode as a function of concentration of KCl showed no significant changes in band positions or relative intensities, even at supporting electrolyte concentrations comparable to the Ag sol systems.

SERS Spectrum of the Semiquinone Radical. Figure 3A shows a SERS spectrum of FMN obtained with 602.4-nm excitation in pH 4.5 solution at −0.3 V vs. SCE where the flavin reduction occurs. Some new bands appeared with a concomitant intensity decrease of those known bands corresponding to the oxidized flavin when the potential was changed from −0.1 to −0.3 V. To isolate the bands due to the new species, a spectrum obtained at −0.1 V where only oxidized flavin exists was multiplied by a factor of 0.4 so that most oxidized bands have similar intensities with those at −0.3 V. It was then subtracted from Figure 3A, giving the spectrum of Figure 3B attributable to the new species. Since this spectrum disappeared when the potential was more negative than −0.5 V vs. SCE where the flavin is fully reduced in a pH 4.5 solution, it is apparent that fully reduced flavin is not responsible for the new spectrum. It was at first sight surprising that no SERS spectrum of reduced flavin was observed with yellow-red excitation. This observation cannot be explained by the lack of resonance enhancement (reduced flavin is colorless) since the oxidized flavin does not absorb light in this frequency region as well. However, the N_5 and N_1 of the isoalloxazine ring are protonated upon the reduction leading to a nonplanar configuration of the molecule. The loss of both nitrogen lone pairs and conjugated π orbitals, which are generally responsible for the chemisorption of SERS molecules, may account for the absence of the reduced flavin SERS spectrum. Thus the flavin semiquinone radical, the intermediate of a two-step single electron transfer reduction, is proposed for the new spectrum. For comparison, the band positions and relative intensities of Clostridium MP flavodoxin semiquinone in aqueous solution observed by CARS[22] are presented by the straight lines at the bottom of Figure 3.

With the exception of a band at 1532 cm^{-1}, bands in Figure 3B at frequencies above 1000 cm^{-1} (CARS spectrum is not available below 1000 cm^{-1}) correspond to those in the solution

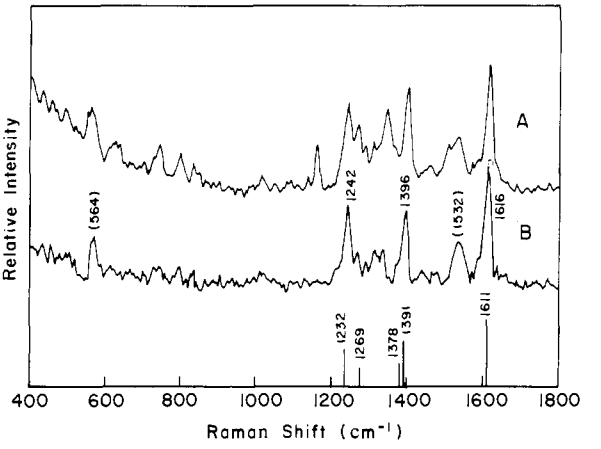

Figure 3. SERRS spectrum of semiquinone flavin obtained in pH 4.5 solution at −0.3 V vs. SCE with excitation frequency of 602.4 nm (A); in spectrum B the Raman bands of the oxidized flavin are subtracted out from spectrum A. The straight lines at the bottom are labels of Raman bands of Clostridium MP flavodoxin in the neutral semiquinone form obtained by CARS.[7]

(20) Abe, M. In *Spectroscopy of Biological Systems*; Clark, R. J. H., Hester, R. E., Eds.; John Wiley & Sons: New York, 1986; Vol. 13, p 347.
(21) Abe, M.; Kyogoku, Y. *Spectrochem. Acta, Part A*, in press.
(22) Dutta, P. K.; Spiro, T. G. *Biochemistry* **1980**, *19*, 1590–1593.

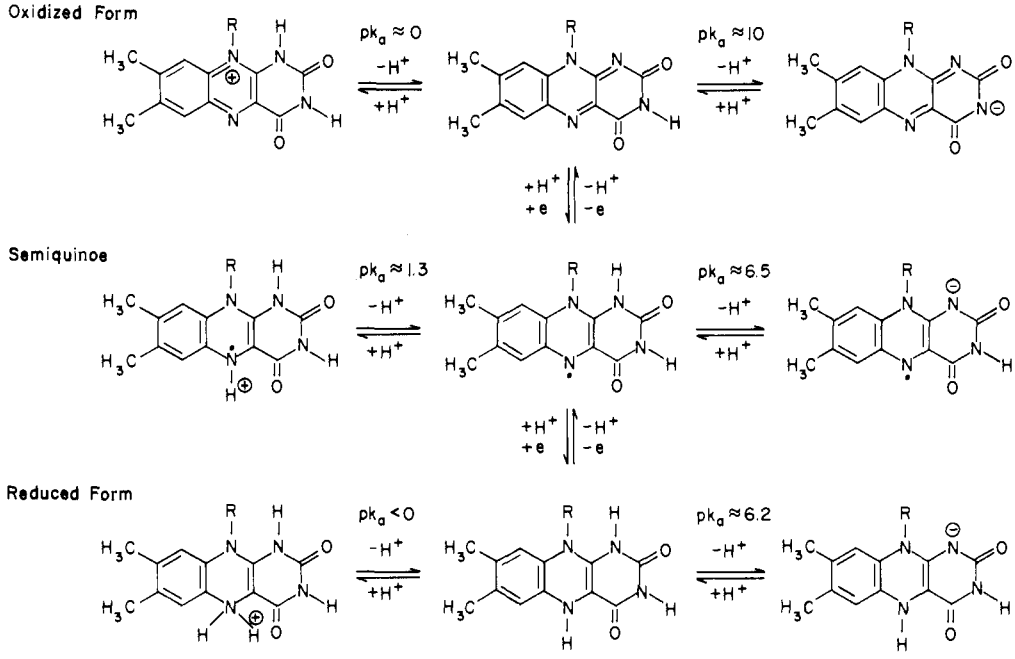

Figure 4. Flavin redox system and occurrence of the flavin species as a function of pH and redox state (adapted in the manner of Dryhurst,[23] from Hemmerich et al.[24] and Janik et al.[26]).

Raman spectrum of the semiquinone with only slight frequency shifts. The relative intensity of the 1532-cm^{-1} band of the oxidized flavin increases at more negative potentials. Therefore, this was not subtracted out as were other bands of the oxidized flavin. Three other bands at 1616, 1396, and 1242 cm^{-1} are identified as surface spectral bands of the neutral semiquinone adsorbed on the Ag electrode (Figure 3B).

Three ionized forms of the semiquinone flavin (anion, neutral, and cation) may exist in solution (Figure 4). The pH range for observing the SERS spectrum of Figure 3B is from 2 to 7, consistent with the acid dissociation property of the flavin semiquinone radical. The pK_a value for the cation is ca. 1.3 and for the neutral radical it is 6.5, so that neutral semiquinone is the major component in the pH 2–7 solution.[23,24] It is known that the neutral flavin radical has a blue color with ϵ_{max} at about 580 nm.[25] Thus, the spectrum obtained with 602.4-nm exciting light (within the absorption band) is actually a SERRS spectrum. In solution a single band at ca. 1616 cm^{-1} is observed with 488-nm laser excitation within the second absorption band of flavin radical (ϵ_{max} is at about 510 nm).[25] However, it does not appear in the surface spectrum excited with 488- or 514.5-nm laser light possibly due to the destabilization of surface semiquinone species by a photoeffect.

At pH above 6.5 (the pK_a value of neutral semiquinone radical), the flavin semiquinone anions are the major species of the radical intermediate. In repeated attempts we could not observe any new bands at flavin reduction potentials from −0.5 to −0,7 V in pH 8–12 solutions with excitation frequencies from 488 to 647.1 nm. The SERS spectrum from oxidized flavin disappears at potentials more negative than −0.7 V vs. SCE. It should be noted that this potential is well positive to the potential at which the loss of SERS active sites begins. Thus no evidence for the existence of semiquinone anion intermediate was observed by SERS.

Electron-Transfer Process of Flavin Reduction on a Silver Electrode. Cyclic voltammetry (CV) was carried out in 1 mM FMN and 0.1 M K_2SO_4 solution with pH values in the range 2–12. Three typical cyclic voltammetric curves obtained on a SERS pretreated (rough) silver electrode at various pH values are shown in Figure 5. At pH 4.2–4.6, there are two distinguishable waves in the CV curve (Figure 5B). The peak currents are proportional to the square root of the sweep rate (4–400 mV/s), indicating that both waves are diffusion controlled and thus a two-step one-electron-transfer process is suggested. At pH 2–4.2 (Figure 5A), the CV curve is similar to the case of two single-electron transfers with overlapped potentials.[16] At pH above 4.6, the single peak in the CV curves is quite broad probably due to the mixing of multiple processes since the neutral and anion form of the redox species have different reduction potentials.

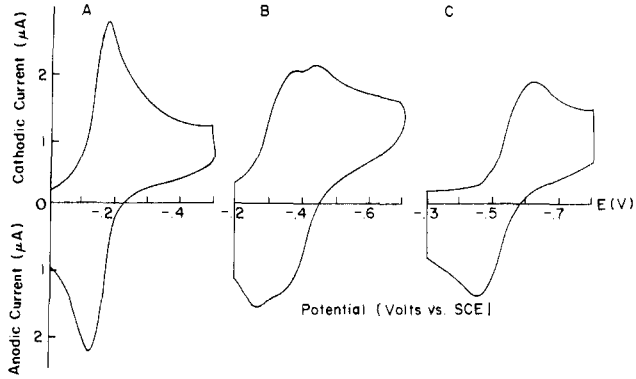

Figure 5. Cyclic voltammetric curves for FMN (1 mM) on a rough Ag electrode with 0.1 M K_2SO_4 as the electrolyte: (A) pH 2.0; (B) pH 4.5; (C) pH 9.0. The scan rate is 20 mV/s.

The observation of the semiquinone intermediate in pH 2–7 solution by SERRS spectra enabled us to conclude that the flavin is electrochemically reduced on a rough silver electrode via two one-electron steps in acidic solution although the potential separation is rather small or even overlapped. Both a polished (smooth) Ag electrode and a pretreated (rough) Ag electrode show CV curves with two diffusion-controlled waves at pH 4.5. This is consistent with the formation of the semiquinone intermediate on the smooth as well as on the rough Ag electrode. The similarity of the dual CV peaks at pH 4.5 on both types of Ag electrodes shows that the same reduction mechanism occurs at the two electrode surfaces. This CV morphology only occurs over the narrow pH range of 4.2–4.6 where the most intense semiquinone spectrum is observed. Much weaker spectra for the semiquinone intermediate are found in the pH 2–4 and 5–7 regions where the

(23) Dryhurst, G. In *Electrochemistry of Biological Molecules*; Academic: New York, 1977; p 371.
(24) Hemmerich, P.; Veeger, C.; Wood, H. C. S. *Angew. Chem., Int. Ed. Engl.* **1965**, *4*, 467.
(25) Dutta, P. K.; Spencer, R.; Walsh, C.; Spiro, T. G. *Biochim. Biophys. Acta* **1980**, *623*, 77–83.
(26) Janik, B.; Elving, P. J. *Chem. Rev.* **1968**, *68*, 295.

CV curves only show one peak. Although the spectral data do not prove a two-step charge-transfer mechanism in these pH regions for the smooth Ag electrode, the fact that the SERS and CV curves establish the existence of the intermediate strongly suggests that a two-step mechanism involving overlapped reduction potentials takes place on both types of electrode surface over the entire pH 2-7 region.

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Photochemistry of Colloidal Semiconductors. 20. Surface Modification and Stability of Strong Luminescing CdS Particles

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Abstract: The preparation of CdS sols with a mean diameter between 40 and 60 Å and a relatively narrow size distribution is described. The colloids could be separated as solids, which in turn could be redissolved to give solutions of some 10^{-2} M. Activation of the particles by a cadmium hydroxide precipitate yielded fluorescing samples with a quantum yield exceeding 50%. The blue or green fluorescence occurred close to the band gap energy, which depended on the size of the particles. Violet fluorescing samples of activated ZnS-CdS co-colloids were also prepared. Photoanodic corrosion measurements showed that the activated CdS colloid was 2000 times more stable than the nonactivated one. Under laser illumination the particles became a little larger which is ascribed to an acceleration of Ostwald ripening due to local heating. Extremely intense laser light decomposed the particles into Cd + S.

In the first papers of this series it was shown that colloidal cadmium sulfide in aqueous solution fluoresces and that this fluorescence is efficiently quenched by certain solutes.[1,2] A large number of reports on the fluorescence of colloidal CdS have appeared in the meantime, including both continuous illumination and laser-flash studies.[3-6] The fluorescence is produced upon the recombination of the charge carriers which are generated by light absorption. In most of these studies, no systematic preparative investigations were made to obtain samples having a high quantum yield of fluorescence. In fact, the quantum yield generally was even below 1%, the reason being that the colloidal particles prepared had a lot of defect sites where radiationless recombination of the charge carriers occurred. The environment also affects the fluorescence. For example, CdS in a dry Nafion film luminesces more strongly than in a wet one.[5a]

If the defect sites are located at the surface of the colloidal particles, there appears to be a chance to influence these sites chemically. In fact, it was recently reported in two studies that the fluorescence intensity may be drastically increased by certain surface modification procedures such as exchanging the aqueous solvent by alcohol,[7,8] covering the surface with cadmium hydroxide or silver sulfide,[8] and adsorbing triethylamine.[7] These procedures may also lead to changes in the shape of the fluorescence spectrum, i.e., to changes in the color of the emitted light. The reader may be reminded that the fluorescence color can also be varied by using sols of different particle sizes[9-12] provided that one is operating in the range of extremely small particles where quantization effects due to the spacial confinement of the charge carriers occur.[13,14] These various procedures offer a great variety of possibilities to prepare and investigate luminescing CdS samples having different properties. In fact, one may even ask the question whether inorganic colloids could be substituted for organic dyes in certain electrooptical devices.

In the present paper, detailed descriptions are given for the preparation of strongly luminescing sols of Q-CdS (Q indicates particles showing size quantization effects) and of co-colloids of CdS and ZnS. These samples have fluorescence quantum yields greater than 50%, and another outstanding feature is the relatively narrow width of the fluorescence band. The stability of these colloids was tested in photoanodic dissolution experiments with continuous illumination and high laser intensity irradiation. Fluorescence lifetime and quenching experiments were also carried out.

Experimental Section

Preparation of CdS Colloids. The strong luminescing colloids were prepared in two steps. First, a base sol was made by precipitating Cd^{2+} ions with the stoichiometric amount of injected H_2S. Secondly, the sol was activated by adding first NaOH and then excess Cd^{2+} ions. The base sol had a weak broad fluorescence band between 500 and 700 nm. The activated sol had a very strong fluorescence band close to the onset of absorption. This band was 10 to 100 times stronger than that at the

(1) Henglein, A. *Ber. Bunsenges. Phys. Chem.* **1982**, *86*, 301-305.
(2) Henglein, A. *J. Phys. Chem.* **1982**, *86*, 2291-2293.
(3) (a) Rossetti, R.; Brus, L. *J. Phys. Chem.* **1982**, *86*, 4470-4472. (b) Chestnoy, N.; Harris, T. D.; Brus. L. E. *J. Phys. Chem.* **1986**, *90*, 3393-3399.
(4) (a) Duonghong, D.; Ramsden, J. J.; Grätzel, M. *J. Am. Chem. Soc.* **1982**, *104*, 2977-2985. (b) Ramsden, J. J.; Grätzel, M. *J. Chem. Soc., Faraday Trans. 1* **1984**, *80*, 919-933. (c) Ramsden, J. J.; Webber, S. E.; Grätzel, M. *J. Phys. Chem.* **1985**, *89*, 2740-2743. (d) Serpone, N.; Sharma, D. K.; Jamieson, M. A.; Grätzel, M.; Ramsden, J. J. *J. Chem. Phys. Lett.* **1985**, *115*, 473-476.
(5) (a) Kuczynski, J. P.; Milosavljevic, B. H.; Thomas, J. K. *J. Phys. Chem.* **1983**, *87*, 3368-3370; **1984**, *88*, 980-984. (b) Kuczynski, J.; Thomas, J. K. *J. Phys. Chem.* **1983**, *87*, 5498-5503.
(6) Tricot, Y.-M.; Fendler, J. H. *J.* Phys. *Chem.* **1986**, *90*, 3369-3374.
(7) Dannhauser, T.; O'Neil, M.; Johansson, K.; McLendon, G. *J. Phys. Chem.* **1986**, *90*, 6074-6076.
(8) Spanhel, L.; Weller, H.; Fojtik, A.; Henglein, A. *Ber. Bunsenges. Phys. Chem.* **1987**, *91*, 88-95.
(9) Fojtik, A.; Weller, H.; Koch, U.; Henglein, A. *Ber. Bunsenges. Phys. Chem.* **1984**, *88*, 969-977.
(10) Henglein, A. In *Modern Trends of Colloid Science in Chemistry and Biology*; Eicke, H.-F., Ed.; Birkhäuser Verlag: Basel, 1985; pp 126-147.
(11) Henglein, A.; Fojtik, A.; Weller, H. *Ber. Bunsenges. Phys Chem.* **1987**, *91*, 441-446.
(12) Baral, S.; Fojtik, A.; Weller, H.; Henglein, A. *J. Am. Chem. Soc.* **1986**, *108*, 375-378.
(13) Brus, L. E. *J. Chem. Phys.* **1983**, *79*, 5566-5571; **1984**, *80*, 4403-4409; **1986**, *90*, 2555-2560.
(14) Weller, H.; Schmidt, H. M.; Koch, U.; Fojtik, A.; Baral, S.; Henglein, A.; Kunath, W.; Weiss, K.; Dieman, E. *Chem. Phys. Lett.* **1986**, *124*, 557-560. Schmidt, H.-M.; Weller, H. *Chem. Phys. Lett.* **1986**, *129*, 615-618.

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