trend-setting term in eq 14. We find in fact, in agreement with experiment,[26a] that $PA[Mn(CO)_5^-] > PA[Co(CO)_4^-]$, and we note that this order for the proton affinities is caused by $Co(CO)_4$ having a larger electron affinity than $Mn(CO)_5$ (Table VI). The proton affinities of $Tc(CO)_5^-$ and $Re(CO)_5^-$ are likewise seen to be larger than the proton affinities for $Rh(CO)_4^-$ and $Ir(CO)_4^-$, respectively, as a result of $Rh(CO)_4$ and $Ir(CO)_4$ having larger electron affinities than $Tc(CO)_5$ and $Re(CO)_5$, respectively. The increase in electron affinity as we move within the same transition series from $M(CO)_5$ with a metal center from the middle of the series to $M(CO)_4$ with a metal center from the end of the series correlates with a general decrease in the energy of *n*d toward the end of the three transition series. We expect within a given transition series ML_n^- of the early or middle elements to have a larger proton affinity than ML_n^- made up of the late elements for the "same" set of ligands.

The electron affinities of $M(CO)_5$ (M = Mn, Tc, Re) were calculated as the energy difference between $M(CO)_5^-$ of bipyramidal geometry and $M(CO)_5$ with the geometry of the $M(CO)_5$ framework in **12**. The electron affinities of $M(CO)_4$ (M = Co, Rh, Ir) were calculated as the energy difference between $M(CO)_4^-$ with a tetrahedral geometry and $M(CO)_4$ with the same geometry as the $M(CO)_4$ framework in **13**.

We have not studied in any great detail how other coligands than CO might modify $D(H-ML_n)$ and $PA[ML_n^-]$. However, substituting the carbonyl trans to the hydride in $HCo(CO)_4$ by the better σ-donor and poorer π-acceptor PH_3 to produce $HCo(CO)_3(PH_3)$ does only reduce the homolytic M-H bond strength by 11 kJ mol^{-1}, whereas $PA[Co(CO)_3PH_3^-]$ is seen to be 58 kJ mol^{-1} smaller than $PA[Co(CO)_4^-]$ (Table VI). The reduction in the proton affinity caused by the phosphine substitution stems from $Co(CO)_3PH_3$ having a lower electron affinity than $Co(CO)_4$ (Table VI), as a result of the electron accepting metal (s,p,d)-hybride being more antibonding in $Co(CO)_3PH_3$ than in $Co(CO)_4$.[27b]

Our calculated value for $PA[Mn(Co)_5^-]$ of 1253 kJ mol^{-1} compared reasonably well with $PA[Mn(CO)_5^-] = 1330$ kJ mol^{-1} obtained experimentally by Stevens and Beauchamp.[28] We have in a previous study[27b] calculated the proton affinity of $M(CO)_5^-$ (M = Mn, Tc, Re) and $M(CO)_4^-$ (M = Co, Rh, Ir) using the HFS method. The trends in the PAs are the same in the two studies, although the PAs calculated by the present method are some 35 kJ mol^{-1} higher than the PAs evaluated by the HFS method.

VIII. Concluding Remarks

We have studied the homolytic M-H and $M-CH_3$ bond energies in MX^{n+} and $XM(CO)_m^{n+}$ of middle to late transition metals. We have found for the neutral molecules with $n = 0$ that the $M-CH_3$ bond is weaker than the M-H bond, in spite of the fact that $M-CH_3$ is stabilized relative to M-H by a stronger σ-bonding interaction as well as charge transfer from the occupied π_{CH_3} orbitals to empty nd_π or $(n+1)p_\pi$ metal orbitals, as a result of destabilizing exchange repulsions between the fully occupied $1\sigma_{CH_3}$ orbital and occupied metal orbitals. For the positively charged molecules with $n = 1$ the charge transfer from CH_3 to the metal center is enhanced and the exchange repulsions are reduced due to a contraction of the metal orbitals in the presence of the positive charge. The strength of the $M-CH_3$ bond is as a result increased to the point where the $M-CH_3$ bond becomes stronger than or as strong as the corresponding M-H bond. We have finally given an analysis of the factors responsible for periodic trends in the proton affinities $PA[M(CO)_m^-]$ of $M(CO)_m^-$. It is shown that the homolytic bond energies $D(H-M(CO)_m)$ of $HM(CO)_m$ as well as the electron affinities $A[M(CO)_m]$ of $M(CO)_m$ are responsible for the trends (increase) in $PA[M(CO)_m^-]$ down a triad, whereas $A[M(CO)_m]$ is responsible for the trends (decrease) along a period.

The calculations presented here are based on a relatively new[9] but well tested[9,14c] density functional method. We expect the bond energies obtained here to be accurate to within 50 kJ mol^{-1} or less, with an even smaller error margin for the difference $D(H-M) - D(CH_3-M)$. We do not expect the conclusions drawn here to be changed by calculations based on extensive configuration interaction methods or more accurate density functionals.

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Reinterpretation of Surface-Enhanced Resonance Raman Scattering of Flavoproteins on Silver Colloids

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Abstract: The surface-enhanced resonance Raman scattering of riboflavin binding protein, glucose oxidase, lactate oxidase, *p*-hydroxybenzoate hydroxylase, Old Yellow Enzyme, and flavodoxin (*M. Elsdenii*) on colloidal silver has been investigated. The signals are shown to arise from free flavin extracted from the proteins. No spectra of flavins incorporated in proteins are observed.

Application of surface-enhanced Raman scattering (SERS) to proteins and surface-enhanced resonance Raman scattering (SERRS) to chromophores imbedded in proteins is under extensive investigation.[1] The application to small molecules of biological interest is now well-established.[2] Silver electrodes and colloidal

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silver have been employed. These studies generally exploit the high sensitivity of SERS and the quenching of fluorescence caused by adsorption of molecules on metallic surfaces. The conclusions do not depend upon those aspects of SERS theory which are still in dispute.

Resonance Raman spectroscopy (RRS) is now well-established[3] as a tool for the elucidation of the structure of flavins and flavoproteins. For example, RRS can reveal subtle details of hydrogen bonding in flavoproteins,[4] charge-transfer interactions between flavins and the substrates or inhibitors of the reactions catalyzed by flavoproteins,[5] and the presence of semiquinone intermediates.[6]

Flavin RRS is plagued by the intense fluorescence of the isoalloxazine moiety. The fluorescence is intense enough to completely obscure the resonance Raman spectra of free flavins and many flavoproteins. Almost every known approach to fluorescence rejection and fluorescence quenching has been applied to flavin RRS.

Nishimura and Tsuboi[7] demonstrated that FMN and FAD RRS could be obtained by excitation into the second or third electronic transition. Spiro and co-workers introduced the use of coherent Raman spectroscopy to reject flavin fluorescence.[8] Nishina and co-wokers[9] studied nonfluorescent proteins. McFarland and co-workers relied upon the use of collisional quenchers.[4b] Recently, Sugiyama and co-workers have demonstrated that in some cases signal averaging and background subtraction is adequate.[10] Each of these experimental techniques has proven useful.

Copeland and co-workers introduced the use of SERRS to the study of flavoprotein Raman spectroscopy.[1c] They demonstrated high signal/noise ratio Raman spectra from submicromolar solutions of glucose oxidase (GO) and riboflavin binding protein (RBP), employing colloidal silver as the surface-enhancing medium.

Ring III modes of SERRS spectra of flavins were shown by Copeland et al.[1c] to be significantly (up to 20 cm^{-1}) shifted from their frequencies in free flavins. These shifts were interpreted as evidence for adsorption of the flavin to silver through N(3). That direct contact between N(3) and silver is required for SERRS at colloidal silver has been demonstrated by experiments showing that N(3)-substituted flavins do not exhibit SERRS spectra.[1d] That the active species is the N(3) anion is confirmed by the pH dependence of the spectra and the similarity of spectra in H_2O and D_2O solutions. The SERRS spectra of flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) are identical with that of riboflavin, but are somewhat less intense.

A silver colloid is a complicated system. It is known that colloid coagulation is a required first step for intense surface enhancement. Extended storage of the colloid in the dark, an excess of adsorbates which reduce surface charge, or an excess of salts can all cause coagulation of the colloid.[11] Because the state of the colloid is both time and medium dependent, careful control of conditions is needed to obtain interpretable results.

These complications have been demonstrated in the SERRS of free flavins.[1d] The relative intensities of bands are time dependent, both because of changes in the state of aggregation of the silver colloid and because of photodecomposition with extended exposure to the laser beam. These complications suggest that the SERRS of flavoproteins may be equally or even more complicated than the SERRS of free flavins.

The known three-dimensional structures of flavoproteins all show the flavin to be buried within the protein matrix.[12a-e] This suggests that direct contact between the protein-bound flavin and the colloidal silver surface is unlikely. That flavoproteins,[13] including GO,[14] are not electrochemically reducible without mediators is further evidence for the lack of direct flavin/metal contact. Chemical accessibility studies[12f] of 2-thioflavin reconstituted enzymes indicate that in Old Yellow Enzyme (OYE), lactate oxidase (LO) and flavodoxin (FD), the C(2)–N(3) region is buried within the protein matrix. For OYE, LO, and FD direct contact between N(3) and an external surface is precluded. For GO direct contact is unlikely.

The behavior of *p*-hydroxybenzoate hydroxylase (PHBH) and riboflavin binding protein (RBP) is more subtle. In both cases chemical accessibility studies demonstrate that C(2)=O is accessible to attack by reagents in solution.[12f] However, the crystal structure of PHBH demonstrates that the flavin is buried within the protein and that solvent accessibility is via channels into the protein.[12e] In addition, the N(3) position is hydrogen-bonded to the protein matrix. The structure of RBP is not known and it is possible, although not proven, that the N(3) position may be on the surface of the protein and not merely accessible to the solvent.

We therefore decided to reinvestigate the SERRS of flavoproteins. We have chosen to repeat the studies of Copeland and co-workers and to extend them to a series of proteins for which there is structural or chemical evidence on the accessibility of the N(3) position. Accordingly we have reinvestigated the SERRS of GO and RBP on colloidal silver and report the SERRS from four other flavoproteins. These are flavodoxin from *M. Elsdenii* (FD), LO, PHBH, and OYE. All six proteins have been extensively investigated by resonance Raman[3a,b] or resonance nonlinear Raman[3d,4a,8a] spectroscopies. For each protein, spectra are available at or near pH 7.2, the pH of our experiments. In some cases confirming spectra are available from two or more laboratories.

Experimental Section

Riboflavin, FMN, and FAD were purchased from Sigma. Lumiflavin was a generous gift from the late Dr. Peter Hemmerich, University of Konstanz, W. Germany. FMN was purified[15] by adsorption onto an

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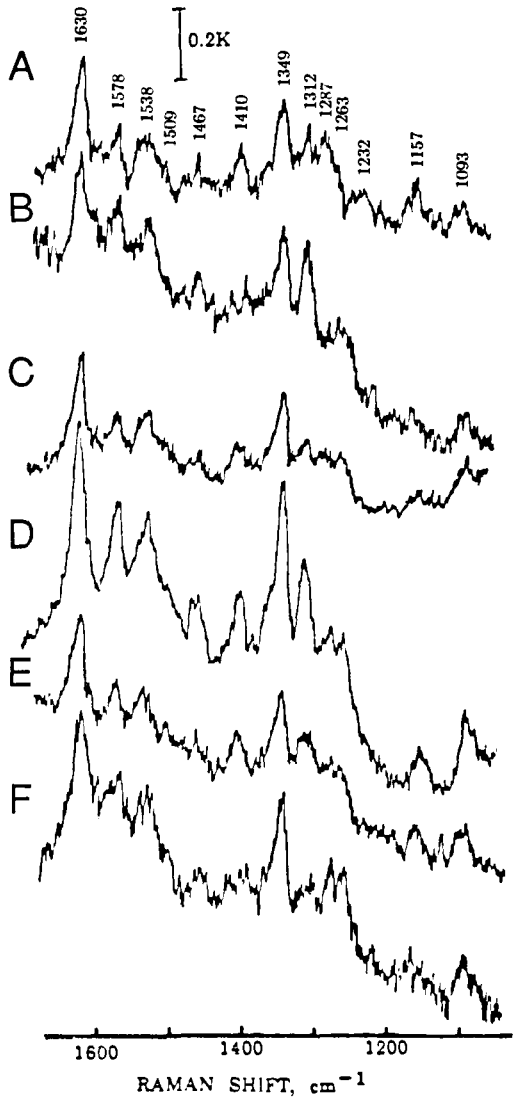

Figure 1. SERRS from flavoproteins: (A) riboflavin binding protein, (B) glucose oxidase, (C) flavodoxin from *M. Elsdenii*, (D) lactate oxidase, (E) *p*-hydroxybenzoate hydroxylase, (F) Old Yellow Enzyme. Conditions: flavin concentration, 1×10^{-6} M; pH 7.2; laser power, 20 mW, 488 nm; resolution, 9 cm^{-1}; scan speed, 100 cm^{-1} min^{-1}; scan time, 7 min; silver sol concentration, 5×10^{-4} M.

apo-flavodoxin column followed by elution and concentration. Riboflavin binding protein,[16] glucose oxidase,[17] *M. Elsdenii* flavodoxin,[18] lactate oxidase,[19] *p*-hydroxybenzoate hydroxylase,[20] and Old Yellow Enzyme[21] were purified as previously described. The enzymes were passed over Sephadex G-25 equilibrated in distilled water immediately before combining with the silver sol in order to remove the traces of free flavins which frequently result from storage and handling of flavoproteins. The following proteins were used as received: RNA-ase from bovine pancreas (Sigma, Type 1A), lysozyme from hen egg white (Sigma, Grade 1), soybean trypsin inhibitor (Sigma, Type 1S), and DNA-ase (Worthington). All other reagents were ACS Reagent Grade and were used as received. Type I water was used to prepare all solutions. Silver colloids were prepared by the EDTA reduction procedure previously described.[22]

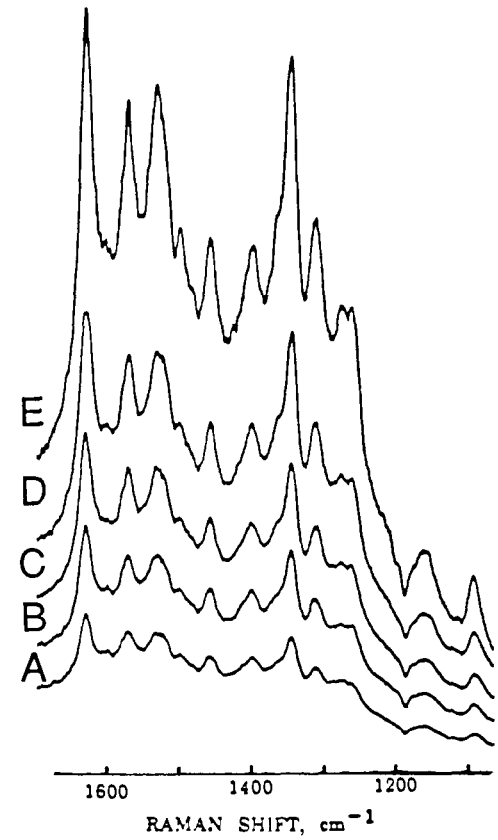

Figure 2. SERRS spectra of 1×10^{-6} M glucose oxidase taken immediately after mixing with 5×10^{-4} M silver colloid. Conditions: pH 7.2; laser power, 30 mW, 488 nm; resolution, 5 cm^{-1}; laser irradiation time: (A) 50 s, (B) 90 s, (C) 140 s, (D) 200 s, and (E) 450 s.

In general, solutions were not buffered except by the buffering capacity of the EDTA remaining in the colloid dispersion. The pH of the sols was brought into the range 7.2–7.3 by addition of HNO_3 as needed.

Raman spectra were obtained with argon ion 488-nm excitation. Laser power was limited to 10–30 mW at the sample. The Raman system consisted of a Spex 1401 monochromator with cooled RCA C31034 photomultiplier and photon-counting electronics. Spectra were acquired at 100 cm^{-1}/min with 9-cm^{-1} resolution. Alternatively, a triple monochromator, Spex 1877, equipped with a Tracor Northern image-intensified diode array was used to obtain spectra with short and precisely known laser exposure. Spectra were acquired with 10-s integration times. For most experiments, samples were contained in 1.5-mm i.d. melting point capillaries. Flow experiments employed a section of 1.5-mm i.d. capillary tubing and a peristaltic pump providing 4 mL/min recirculating flow from a reservoir containing about 5 mL.

Results and Discussion

SERRS spectra from the six flavoproteins (RBP, GO, FD, LO, PHBH, and OYE) are shown in Figure 1. The spectra were obtained on freshly purified samples within 1 h after mixing colloid with each protein. The spectra are remarkable in that they are at least 50 × less intense than FMN or FAD spectra obtained under the same conditions. Wherever they can be measured above the system noise, the bands occur at substantially the same frequencies in each system, as shown in Table I. By contrast, the Raman band positions of the same flavoproteins vary substantially, as shown in the table. For example, the frequency of band I varies from about 1621 cm^{-1} in PHBH to 1637 cm^{-1} in LO. Similarly, the band II varies from 1580 cm^{-1} in FD to 1594 cm^{-1} in LO. In the SERRS spectra band I is unchanged (± 1 cm^{-1}) throughout the series of flavoproteins, and band II varies over a range of 2 cm^{-1}. The pattern is repeated throughout the spectra. All of the SERRS spectra are similar to those of free flavins, such as RF, FMN, or FAD. However, they are not exactly identical with those of free flavins. This point will be addressed below.

One implication of the data of Figure 1 is that the flavoprotein spectra might arise from free flavins formed by dissociation of

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Table I. Observed Raman Frequencies (cm^{-1}) for Flavoproteins: from SERRS, RR, CARS, and IRS[a]

bands[b]	SERR (RR)[c] RBP	SERR (CARS)[d] GO	SERR (CARS)[d] FD	SERR (CARS)[d] LO	SERR (IRS)[e] PHBH	SERR (RR)[f] OYE
	1630	1630	1629	1631	1630	1630
I	(1631)	(1631)	(1630)	(1637)	(1621)	(1628)
	1578	1578	1579	1579	1580	1579
II	(1582)	(1583)	(1580)	(1594)	(1595)	(1588)
	1538	1537	1538	1537	1539	1538
III	(1547)	(1534)	(1554)	(1561)	(1547)	(1548)
	1509	1508	1510	1510	1510	1510
IV	(1501)	(1505)	(1501)	(1513)	(1508)	(1517)
	1467	1467	1468	1468	1468	1468
V	(1462)	(1467)	(1460)	(1456)	(1468)	(1475)
	1410	1412	1410	1410	1412	1410
VI	(1407)	(1409)	(1410)	(1416)	(1418)	(1411)
	1349	1348	1348	1348	1348	1348
VII	(1354)	(1345)	(1355)	(1366)	(1355)	(1359)
	1312	1310	1313	1313	1313	1310
VIII	(1304)	(xxxx)	(xxxx)	(1307)	(1311)	(1310)
	1287	1282	1282	1281	1281	1281
IX	(1281)	(1284)	(1275)	(xxxx)	(1284)	(xxxx)
	1263	1260	1263	1263	1263	1260
X	(1250)	(1248)	(1257)	(1261)	(1258)	(xxxx)
	1235	xxxx	xxxx	xxxx	xxxx	xxxx
XI	(1228)	(1235)	(1230)	(1234)	(1241)	(xxxx)
	xxxx	xxxx	xxxx	xxxx	xxxx	xxxx
XII	(1178)	(1185)	(1176)	(1191)	(1195)	(1194)
	1157	xxxx	xxxx	1156	1156	1156
XIII	(1161)	(1165)	(1165)	(1173)	(1163)	(xxxx)
	1093	1090	1090	1092	1093	1093
	(1072)	(xxxx)	(xxxx)	(xxxx)	(xxxx)	(xxxx)

[a] Abbreviations: RBP, riboflavin binding protein; GO, glucose oxidase; FD, flavodoxin; LO, lactate oxidase; PHBH, *p*-hydroxybenzoate hydroxylase; OYE, Old Yellow Enzyme; SERR, surface-enhanced resonance Raman; RR, resonance Raman; CARS; coherent antistoke Raman scattering; IRS, inverse Raman scattering. [b] Band numbering scheme of: Bowman, ; Spiro, T. G. *Biochemistry* **1981**, *20*, 3313–3318. [c] From ref 3b. [d] From ref 8a. [e] From ref 4a. [f] From ref 5c.

flavoproteins, or by decomposition during the Raman experiments. Therefore, we have investigated the time dependence of flavoprotein SERRS in more detail.

Figure 2 shows the effect of exposure to laser irradiation on freshly prepared GO mixed with colloidal silver and introduced into the spectrometer within 5 min after mixing. Spectra were taken with the array detector system, with 10-s exposure time. The listed times are the total irradiation times at the end of each exposure. The SERRS spectrum is initially weak but continues to increase in intensity with time.

Figure 3 shows the time effects of exposure to laser irradiation on freshly prepared GO mixed with colloidal silver and stored in the dark for 24 h before exposure to laser irradiation. These conditions are similar to those employed by Copeland et al. Again, with increasing exposure to laser light, the spectra increase in intensity. With increasing time, they become similar to the spectra of GO previously reported by Copeland et al.[1c]

The data in Figures 2 and 3 demonstrate that exposure to colloidal silver is not sufficient to cause the observed changes. Rather, the system must be exposed to laser light. There are no major changes in band positions or relative band intensities during the time sequence experiments. The increasing intensity could be caused either by increasing FAD concentration from laser-induced protein decomposition, or by changes in the state of aggregation of the colloid, or both.

To examine the effect of proteins on colloid aggregation, we studied solutions containing 1×10^{-6} M RBP or GO and 10×10^{-6} M RNA-ase, lysozyme, DNA-ase, or soybean trypsin inhibitor. None of these proteins binds flavins. If they compete with the flavoproteins for surface sites, or otherwise affect the colloid, there should be measurable effects on the SERRS of flavoproteins. In each case the behavior was the same as observed for the flavoprotein alone under the same conditions of time and laser exposure. The variety of proteins tested suggests that competition between proteins for the silver or protein-induced changes in the colloid cannot explain our results.

That the SERRS spectra from flavoproteins is due to free flavins is confirmed by experiments on flavoprotein/apo-RBP mixtures. A typical example, flavodoxin/apo-RBP is summarized in Figure 4. Trace A shows the SERRS spectrum of FMN, 1×10^{-6} M. Trace B shows the spectrum obtained by scanning a freshly prepared colloid/FD sample. The spectra are similar, but the intensities are about 50× lower than observed for free FMN at the same concentration. Trace 4C shows the results of a scan obtained from a freshly prepared mixture of 1×10^{-6} M apo-RBP and FD to which colloid had been added. If, as we propose, the SERRS spectrum is due to 1–2% of dissociated FMN, the apo-RBP concentration represents a 50-100-fold excess over that of the free flavin. This excess should be sufficient to bind substantially all of the free FMN. No SERRS bands are visible in this spectrum.

The addition of apo-RBP causes the disappearance of the spectra of any of the flavoproteins. Because spectra from flavoproteins were initially weak, we conclude that their intensities must be reduced by at least a factor of 10–50. In control experiments, the SERRS intensities of free FMN or FAD were reduced by a factor of 50–100 by addition of equimolar quantities of apo-RBP.

The necessity of direct contact between the flavin N(3) position and the silver surface[1d] provides a severe constraint for the SERRS spectra of flavoproteins. For four of the flavoproteins studied (OYE, LO, FD, and PHBH), the independent structural evidence is not consistent with SERRS from an N(3)-adsorbed flavin species. SERRS spectra must therefore arise from flavin released from the flavoprotein. The electrochemical behavior of GO[14] makes valid SERRS from the intact enzyme improbable. That conclusion is confirmed by our results. Taken together, the weight of our observations is that the spectra attributed to both GO and RBP are also actually due to free flavins released from these flavoproteins rather than the intact flavoproteins.

The behavior of these systems is further complicated by evidence of photodecomposition of riboflavin to lumiflavin upon laser exposure. Decomposition is apparent in the time sequence experiments on free riboflavin shown in Figure 5. Trace A shows the spectrum of riboflavin taken in a flow cell. This spectrum, obtained during a 7-min scan, could be repeatedly obtained. Trace F shows

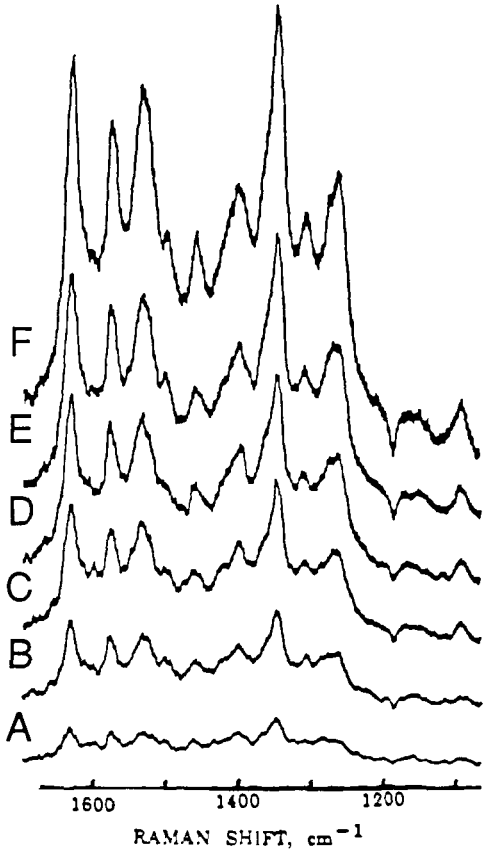

Figure 3. SERRS spectra of 1×10^{-6} M glucose oxidase 24 h after mixing with silver colloid. Conditions, as in Figure 2, except laser power 20 mW, laser irradiation time: (A) 15 s, (B) 35 s, (C) 50 s, (D) 75 s, (E) 110 s, and (F) 150 s.

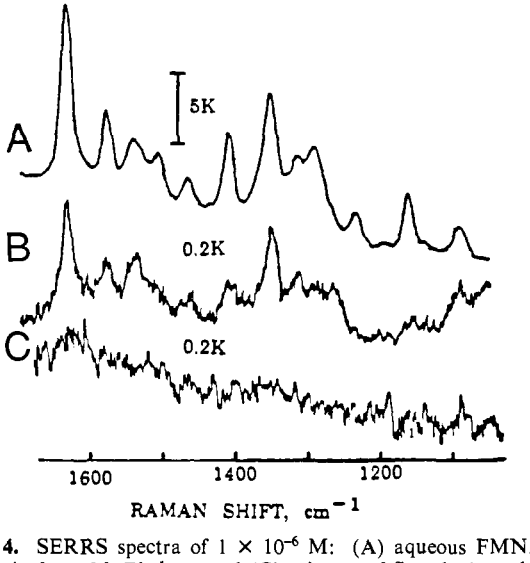

Figure 4. SERRS spectra of 1×10^{-6} M: (A) aqueous FMN, (B) flavodoxin from *M. Elsdenii*, and (C) mixture of flavodoxin and apo-RBP (1:1 ratio). Conditions, as in Figure 1.

the spectrum of lumiflavin, obtained in the flow cell. Again, this spectrum was stable. Traces B–E show the time evolution of the riboflavin spectrum in a static system over a 28-min period. In these spectra the relative intensities of the 1160-cm^{-1} band is decreasing, while the 1260-cm^{-1} band is evolving from a shoulder to a distinct band and the 1429-cm^{-1} band begins to appear. These spectra confirm that decomposition accompanies laser exposure and that reported spectra of riboflavin derivatives may be confounded with signals from lumiflavin photoproducts. Inspection of the spectra in Figures 1–3 suggests that there may be some photodecomposition accompanying release of flavins from flavoproteins.

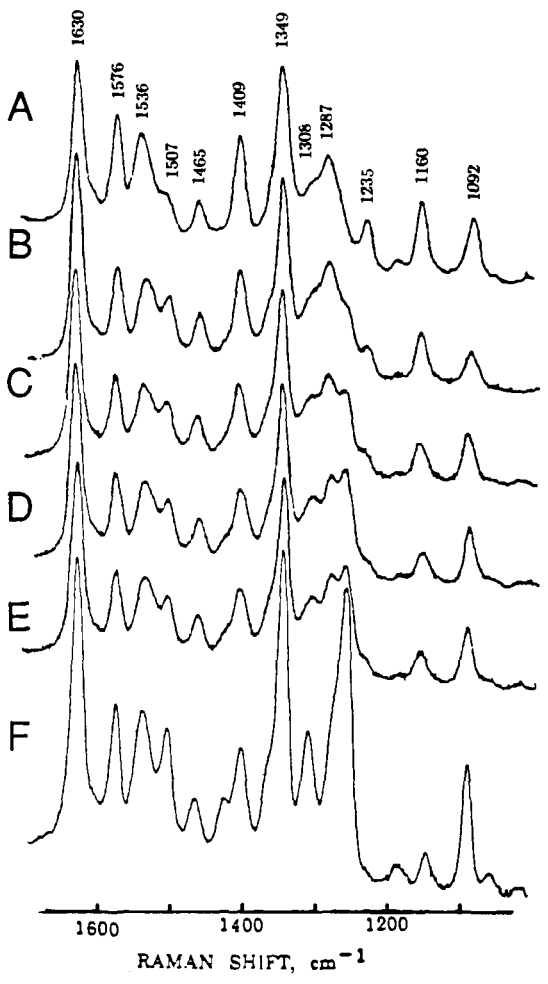

Figure 5. Effect of laser irradiation on SERRS of 1×10^{-6} M riboflavin (RF): (A) RF, in flow cell, 4 mL/min; (B) RF, first scan in static system; (C) RF, second scan; (D) RF, third scan; (E) RF, fourth scan; (F) 1×10^{-6} M lumiflavin in flow cell. Conditions, as in Figure 1, except laser power 10 mW.

All of the flavoproteins in this study are normally in equilibrium with small amounts of free flavin. In addition, they undergo decomposition during storage to release free flavin. To remove free flavin each enzyme was filtered over Sephadex G-25 immediately prior to use. In a stationary sample, the intensity of the resultant SERRS signals was always weakest immediately after filtration, but increased with time. In a flow cell, a carefully purified sample gave no signal. If the sample was slightly decomposed, the initial weak signal did not increase with time. These results suggest that the flavoproteins undergo additional, slow dissociation in the laser beam.

The SERRS spectra obtained from the flavoproteins are not quite identical with the spectra of free flavins. Proteins themselves are known to be adsorbed on silver colloids, and can generate SERS[23] if present at high concentration. Furthermore, the interfacial potential of the silver colloid is not known and can vary depending on the species adsorbed on it. SERRS relative intensities are known to be potential-dependent at silver electrodes. A small amount of decomposition, residual interactions between co-adsorbed protein and flavin, changes in interfacial potential, or any combination can account for the differences observed between the SERRS spectra from the flavoproteins and those of free flavins.

Because total residence time in the laser beam is not always carefully controlled, decomposition appears to be a major source of the observed variability in the spectra of free riboflavin, as well.

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Lumiflavin, the photoproduct of riboflavin, is more stable to light than riboflavin. Accordingly, lumiflavin SERRS spectra show less variability over the time normally required to obtain a Raman spectrum.

Conclusions

We conclude that the previously reported SERRS of flavoproteins do not represent the proteins themselves, but rather their dissociated flavins. Our study includes a selection of flavorpoteins sufficiently varied to suggest that great caution will be necessary in the interpretation of the SERRS spectrum of any flavoprotein. A recent report[1a] of extraction of porphyrins from several heme proteins on colloidal silver suggests that SERS study of protein/small molecule complexes may be generally perilous.

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Determination of the Absolute Configuration of Six-Membered-Ring Ketones by ^{13}C NMR

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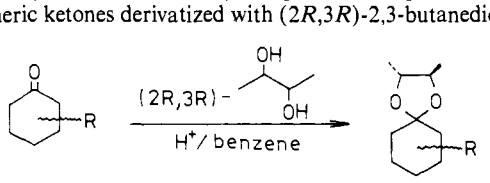
Abstract: The diastereotopic splitting of the ^{13}C NMR signals of the (2*R*,3*R*)-2,3-butanediol acetals of 39 chiral six-membered-ring ketones, including 2- and 3-substituted cyclohexanones, 2-alkyltetrahydropyran-4-ones, and 2- and 3-alkyltetrahydrothiopyran-4-ones, has been studied. The stereodifferentiation of the six-membered-ring carbon atoms was shown to follow a very regular pattern, which is independent of substituents or of heteroatoms in the ring. Hence, an empirical rule could be deduced which allows the assignment of the absolute configuration of the original ketones from the ^{13}C NMR spectrum of their acetals. The rule is only valid for six-membered-ring ketones having the chair form as the preferred conformation in the acetal. As shown for 11 examples, this rule is not valid for acyclic ketones, cyclopentanones, cycloheptanones, or cyclohexanones and cyclohexenones not having the chair form as the preferred conformation. The rule also permits the interpretation of the ^{13}C NMR spectrum of the (2*R*,3*R*)-2,3-butanediol acetals of achiral cyclohexanones (seven examples). The stereodifferentiation is almost exclusively determined by the centers of chirality in the dioxolane ring.

Convenient routes to optically active six-membered-ring ketones have been developed by several research groups. Enantiomerically enriched 2- and 3-substituted cyclohexanones have been obtained from enantioselective reductions of racemic cyclohexanones with HLAD (horse liver alcohol dehyrogenase).[1-5] Cyclohexanones substituted at position 2 have also been synthesized by asymmetric alkylation of cyclohexanone.[6] Optically active 3-(arylthio)- and 3-(arylseleno)cyclohexanones have been obtained from asymmetric conjugate additions of arylthiols and arylselenols to 2-cyclohexenones.[7,8] Optically active 2-alkyltetrahydropyran-4-ones and 2- and 3-alkyltetrahydrothiopyran-4-ones have been obtained from HLAD-catalyzed reduction and oxidation reactions.[9-11] In all these projects the determination of the enantiomeric excess and of the absolute configuration of these ketones is a major element of concern.

The enantiomeric excess has been determined by the method developed by Hiemstra and Wynberg[12] according to which the enantiomeric ketones derivatized with (2*R*,3*R*)-2,3-butanediol into



the corresponding diastereoisomeric acetals. The enantiomeric excess in the original ketones is then obtained by integrating the ^{13}C NMR signals of two diastereotopic carbon atoms of the acetals.

The absolute configuration has been determined by various methods such as octant rule analysis of the Cotton effects and chemical correlation with products of known configuration. It has now been found that the absolute configuration of six-membered-ring ketones can be determined directly from the ^{13}C NMR spectra of the acetals used in the method of Hiemstra and Wynberg.[12] An empirical rule will be formulated.

Discussion

1. Chiral Six-Membered-Ring Ketones. In Tables I and II the ^{13}C chemical shift values (δ) are collected for the six-mem-

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