

In Situ Surface-Enhanced Raman Scattering Studies of the Nitrosyl Adduct of Hemin Adsorbed on Roughened Silver Surfaces in Aqueous Electrolytes

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Modifications in the electrochemical and spectral properties of hemin (Hm) adsorbed on roughened Ag electrodes in an aqueous electrolyte (phosphate buffer, pH 3) induced by brief exposure to mildly acidic solutions containing NO have been examined by in situ surface-enhanced Raman scattering (SERS) using Q band excitation ($\lambda_{\text{exc}} = 532 \text{ nm}$). Two lines of evidence support the formation of the adsorbed nitrosyl adduct of Hm (NO–Hm) under the conditions employed for these experiments: the complete disappearance of the characteristic voltammetric redox peaks of Hm centered at ca. -0.25 V versus SCE, and a significant drop in the intensity, I , of the ring-based ν_{30} mode at 1165 cm^{-1} in the in situ SERS spectrum compared to that of adsorbed Hm under the same conditions. Such a decrease in the intensity of the $I(\nu_{30})$ mode was also found in the solution phase Raman spectrum of Hm in THF and DMF upon NO exposure. Notably absent from the SERS spectrum were features attributable to vibrational modes due to NO and Fe–NO, which are clearly discernible for NO iron macrocyclic adducts in solution phase using Soret band excitation ($\lambda_{\text{exc}} = 413$ and 406 nm). Scanning to sufficiently negative potentials led to the reemergence of the Hm redox peaks and restored the $I(\nu_{30})$ mode in the SERS spectrum recorded at potentials that were positive enough for the reduced form of Hm to undergo full oxidation. These observations clearly indicate that, to the level of sensitivity of these measurements, the formation and subsequent reduction of the adduct can be effected without compromising the integrity of Hm. Careful inspection of the data showed that the SERS spectrum of the NO–Hm adduct resembles that of adsorbed (axially uncoordinated) Hm, rather than its reduced counterpart, measured under otherwise identical conditions, suggesting that the electronic environment within the ring in the NO–hemin adduct and the (NO-free) oxidized Hm are similar. This behavior is in agreement with information derived from Mossbauer effect spectra for NO–Fe macrocyclic adducts.

Introduction

A number of iron macrocycles of the porphyrins and phthalocyanine (Pc) type have been shown to display electrocatalytic properties for the reduction of nitrite in aqueous electrolytes.^{1,2} Much of the experimental evidence points to the formation of a nitrosyl adduct as a key intermediate in the reaction pathway.^{1,2} Efforts in our laboratory have focused on the in situ structural and electronic characterization of two such adduct materials adsorbed on high-area carbon surfaces using synchrotron-based X-ray absorption fine structure (XAFS),^{3,4} [*meso*-tetrakis(*p*-methoxyphenyl)porphyrinato]iron(II), Fe(TMPP), and iron phthalocyanine, FePc. As shown therein, statistical best fits to the extended X-ray absorption fine structure (EXAFS) data placed the Fe center in a pentacoordinated square-pyramidal configuration shifted away from the macrocyclic plane toward the axially bound NO bent at an angle of ca. $30\text{--}40^\circ$ with respect to the normal to that same plane. Further insight into the iron microenvironment in this type of materials, including both Fe–NO and Fe–ring interactions can be gained from the analysis of the vibrational spectra. The present work examines in situ various aspects of the nitrosyl adduct of Hm adsorbed on roughened Ag electrodes using surface-enhanced Raman scattering (SERS) spectra under conditions similar to those employed recently in our laboratory for studies aimed at monitoring the redox and chemical speciation

of the same adsorbed macrocycle as a function of the applied potential and pH. In particular, the exceedingly high quality of the in situ Raman data collected in that investigation made it possible to identify bands characteristic of the reduced and oxidized forms of adsorbed Hm, including its μ -oxo derivative. As will be shown in this work, the in situ SERS spectra of the adsorbed NO–Hm adduct acquired with Q band excitation failed to reveal bands attributed to Fe–N(O) and bound NO, which are commonly found for solution-phase NO macrocyclic adducts using Soret band excitation.^{5–12} Instead, the formation of NO–Hm led to a significant drop in the intensity of the ring-based ν_{30} mode at 1165 cm^{-1} (see below) compared to that of adsorbed Hm under the same conditions, which can then be used as a marker for monitoring the dynamics of the heterogeneous reduction of the adduct in electrochemical environments now in progress in our laboratory.

Experimental Section

Details regarding the procedure employed for the roughening of the Ag electrode, as well as its surface modification with adsorbed Hm (see Scheme 1), were given in an earlier publication¹³ and will not be repeated here. Also provided therein is a description of the spectroelectrochemical cell used for the in situ SERS measurements. Raman spectra were obtained with a Chromex Raman 2000 spectrograph equipped with an Olympus microscope attachment. A 10x lens focused the 532-nm laser (Coherent Verdi V-10) onto a ca. $5\text{-}\mu\text{m}$ spot

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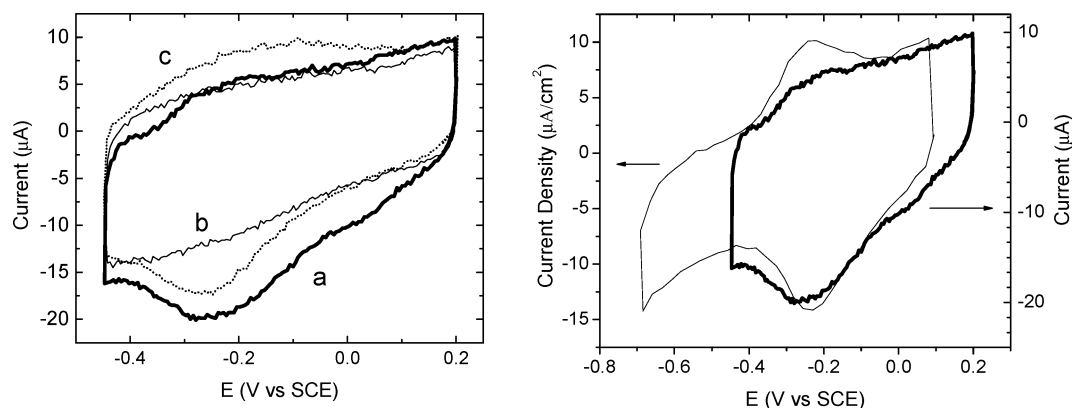
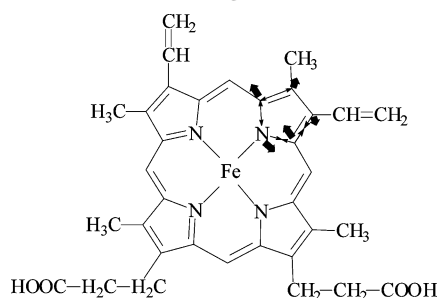


Figure 1. (Left Panel) Cyclic voltammograms for a Hm-modified roughened Ag electrode in neat (NO-free) phosphate buffer, pH 3, before (curve a, dark line) and after exposure to an NO-containing solution (curve b, solid line) in the same electrolyte. (See Experimental Section for details). Curve c in this panel (see dotted line) was recorded following a potential excursion down to -1.2 V vs SCE (a potential sufficiently negative to effect the reduction of NO–Hm adduct) in the same solution. Cross sectional area of the electrode: was 0.28 cm^2 . (Right Panel) Cyclic voltammograms for a Hm-modified roughened Ag (dark curve) and a Hm-modified glassy carbon electrode (light curve).

SCHEME 1: Hemin Showing the ν_{30} Normal Mode



on the electrode surface. The power on the sample was 3 mW. The total acquisition time for each spectrum was 60 s. The electrode potential was controlled by an EG&G PAR 173 potentiostat with a PAR 175 universal programmer.

In situ SERS spectra of adsorbed Hm on roughened Ag were collected in neat deaerated phosphate buffer, pH 3, by scanning the potential to the desired value and waiting for ca. 20 s. The adsorbed Hm–NO adduct was formed in situ by bubbling NO into the solution for about 20 min. The cell was then emptied through a Teflon tubing and immediately refilled with neat deaerated buffer delivered to the cell via a different Teflon tubing without exposing the electrode to the atmosphere.

The Raman spectra of 1 mM deaerated solutions of Hm in tetrahydrofuran (THF, Acros) and *N,N*-dimethylformamide (DMF, Fisher) were recorded in a custom-made Raman cell. The corresponding solution phase NO adducts were prepared following a similar procedure to that reported by Stong et al.,⁹ that is, bubbling NO gas for 20 min and subsequently removing free NO by purging with Ar. The power of the laser on the samples was 4 mW, and the integration time of each of the spectra was 5 min.

Results and Discussion

The cyclic voltammograms of a Hm-modified roughened Ag electrode recorded before (curve a) and after exposure to an NO-containing solution (curve b), in neat (NO-free) phosphate buffer, pH 3, (see Experimental Section) are shown in the Left Panel of Figure 1. As clearly evident from these data, the formation of the NO adduct is accompanied by the disappearance of the redox peaks of Hm. This behavior is in harmony with that found for Hm adsorbed on glassy carbon (see light curve, Right Panel, Figure 1) and also with FeTMPP and FePc adsorbed on high-area carbon electrodes, which is reported in earlier work.³ The much less-defined oxidation feature in the

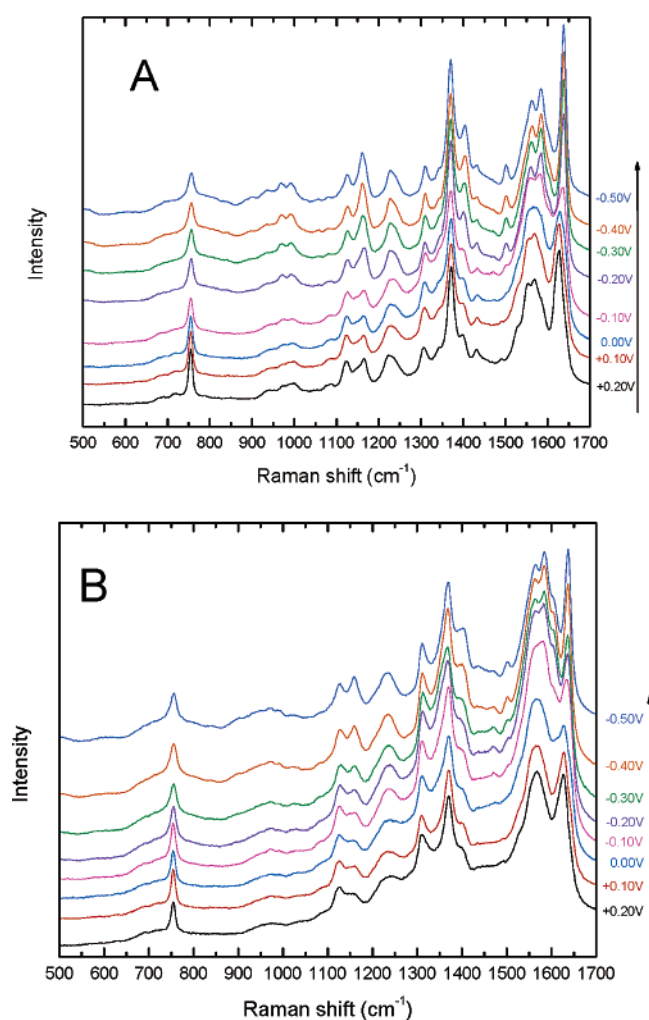


Figure 2. Series of spectra for a Hm-modified Ag electrode in neat phosphate buffer pH 3.0, before (Panel A) and after exposure to the NO-containing solution (Panel B, see text for details) recorded in increments of 0.1 V toward negative potentials in the range $+0.2 \leq E \leq -0.50$ V vs SCE.

scan in the positive direction has also been noted by Rywkin et al. for FeTMPyP adsorbed on roughened Ag electrode in acidic solutions.¹⁴ It must be stressed that both the voltammetric curves and the in situ SERS spectrum obtained for bare roughened Ag (without adsorbed Hm) in the same neat electrolyte were found

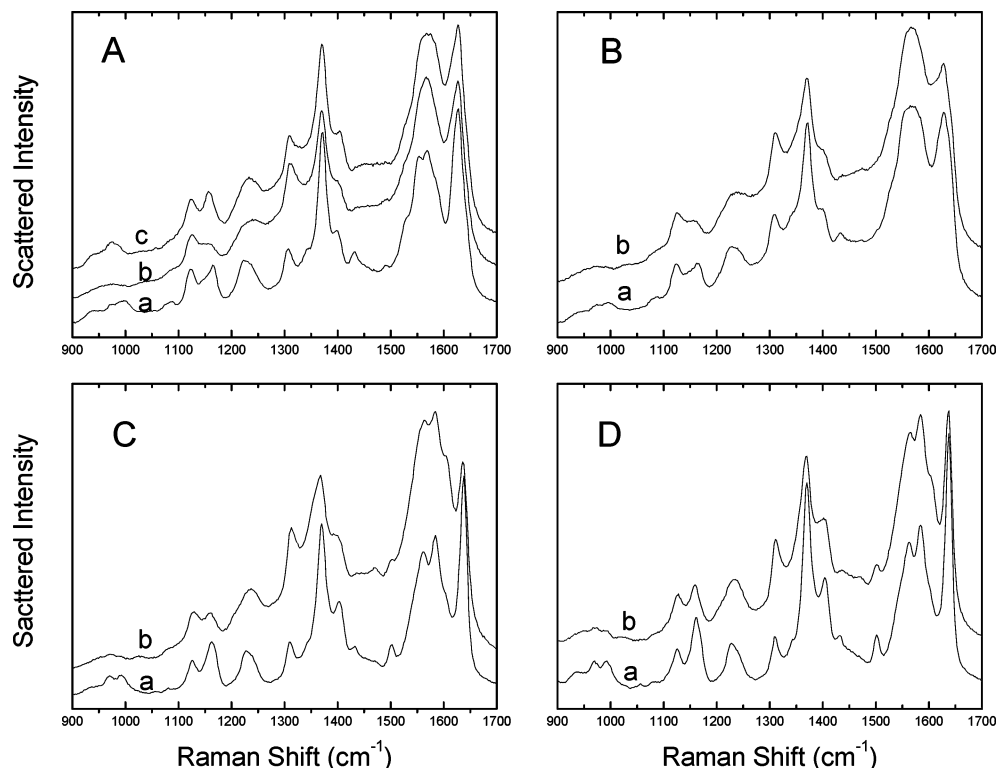


Figure 3. Comparison of spectra recorded for Hm/Ag before (curves a) and after exposure to NO (curves b) at potentials, $E = +0.2$ (Panel A), 0.0 (Panel B), -0.3 (Panel C), and -0.5 V vs SCE (Panel D).

to be featureless over the same potential range both before and after exposure to NO using the same precise protocol (Experimental Section). On the basis of this, it is safe to assume that the electrochemical (and spectroscopic) changes induced by NO exposure of Hm adsorbed on Ag surfaces are due to the binding of NO to adsorbed Hm.

Shown in Figure 2 are a series of spectra for a Hm-modified Ag electrode before (Panel A) and after exposure to the NO-containing solution in neat phosphate buffer (Panel B) recorded in increments of 0.1 V toward negative potentials in the range of $+0.2 \leq E \leq -0.50$ V versus SCE. Notably absent from these spectra are the modes at $1660\text{--}1700\text{ cm}^{-1}$, ascribed to NO (N–O stretching), and Fe–NO (Fe–N stretching) at $515\text{--}530\text{ cm}^{-1}$, reported for NO–Fe porphyrin adducts in nonelectrochemical environments using Soret band excitation ($\lambda_{\text{exc}} = 413$ and 406 nm).^{5–12} Nevertheless, a few significant differences in the spectral features of adsorbed Hm induced by NO binding could be discerned within the voltage range examined, as illustrated for a few selected potential, E , values in Panels A ($+0.2$ V), B (0.0 V), C (-0.3 V), and D (-0.5 V) versus SCE, in Figure 3. These include (a) a marked drop in the intensity, I , of the ν_{30} (B_{2g} 1165 cm^{-1} , Scheme 1), (b) a broadening of the $\nu_5 + \nu_9$ (A_{1g} 1223 cm^{-1}) and ν_4 (A_{1g} 1375 cm^{-1}) modes, (c) an increase in I_{21} (ν_{21} , A_{2g} centered at 1306 cm^{-1}), and (d) the virtual disappearance of the peak centered by 1433 cm^{-1} that is ascribed to the $\delta_s(\text{=CH}_2)$.

It becomes evident from these results that the formation of the axially bound NO–Hm adduct affects modes localized on the chelating ring and that these modified modes can serve as markers for the presence of the adduct at the interface. Indeed, as shown in curve c, Panel A, Figure 3, the spectrum of the interface recorded at $+0.2$ V following excursion to $E = -1.2$ V versus SCE, believed to be negative enough for the complete reduction of the NO-adduct to ensue, resembled rather closely that of Hm at the same potential that was never exposed to NO

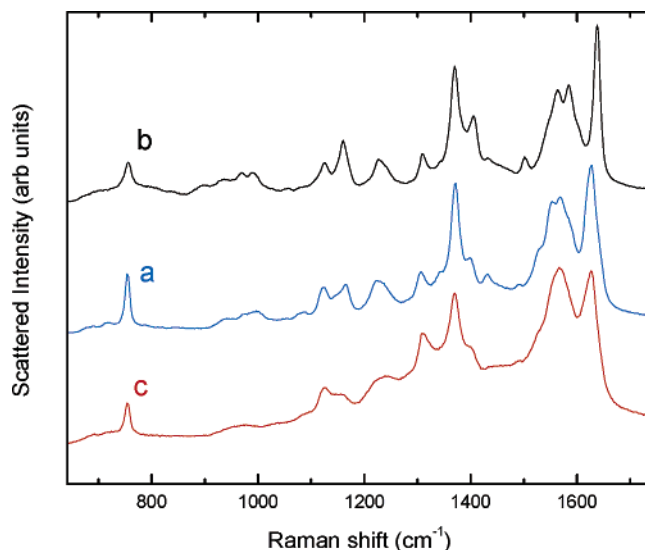


Figure 4. Comparison between the SERS spectra of oxidized ($E = +0.20$ V, curve a) and reduced forms of Hm ($E = -0.55$ V, curve b) adsorbed on a roughened Ag electrode. The corresponding spectrum of Hm–NO recorded at 0.20 V is given in curve c in this figure. All other conditions are specified in the caption Figure 1.

(see curve a in this Figure). This information is consistent with the voltammetric curves recorded following this procedure, which yielded results very similar to that observed prior to NO binding (curve c, Figure 1). The displacement of that curve toward positive currents is most likely due to the (in situ) reduction of traces of dioxygen present in the electrolyte.

As shown in Panel B of Figure 2, the peak centered at 1504 cm^{-1} , characteristic of the reduced form of Hm, becomes clearly visible at ca. -0.20 V, a potential at which the voltammogram affords no evidence for reduction of the NO–Hm adduct. Also noticeable is the slight increase in $I(\nu_{30})$, which

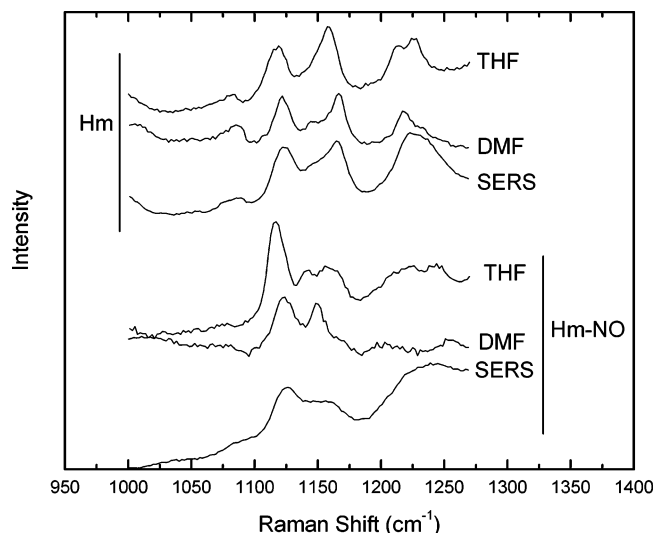


Figure 5. Raman spectra of 1mM solutions of Hm, and Hm-NO in THF and DMF. Also shown for comparison are the SERS spectra obtained in this work in the region of interest.

again points to a decrease in the coverage of the adduct. Because the voltammogram recorded after exposure to NO does not show any evidence for the presence of free Hm, the possibility of an incomplete formation of the NO adduct seems highly unlikely. Also to be considered is a slow reduction of the adduct at potentials more positive than observed within the time scale of the voltammetric measurements (scan rate 50 mV/s). Note that the spectral measurements were performed at fixed potentials (see above) lasting 60 s (plus an additional 20 s to allow for steady-state conditions to be achieved). A more likely explanation for the phenomenon observed is the photoinduced dissociation of the adduct, which has been reported for the homogeneous phase by Hoshino et al.¹⁴ Support for this view was obtained from experiments in which SERS data were collected from +0.2 V down to -0.1 V, that is, slightly negative to the onset of Hm reduction, following the formation of a fresh NO-Hm adduct layer. As evidenced from the data recorded (not shown in this work), a small, but, nevertheless noticeable decrease in I_{22}/I_{30} was observed, pointing to an increase in the amount of (oxidized) Hm. Because of these complications, it was not possible to provide a more quantitative assessment of surface speciation as a function of potential for the NO-Hm adduct.

Further evidence in support of the formation of the NO adduct at the Ag surface was obtained by comparing the solution-phase spectrum of Hm in two nonaqueous solvents in which Hm

displays high solubility, that is, THF and DMF. As shown in Figure 5, the Hm-NO adduct displayed a diminished $I(\nu_{30})$ feature in both solvents by direct analogy to the behavior found on the Ag surface.

As a final point, it is interesting to compare the spectra of both oxidized and reduced forms of adsorbed Hm and the corresponding Hm-NO complex. As shown in Figure 4, the SERS spectrum of the adduct resembles the oxidized form of Hm much more than its reduced counterpart, suggesting that the environment of the ring in the NO-hemin adduct and the (NO-free) oxidized hemin are similar. This behavior is in agreement with the Mossbauer effect spectrum (MES) for NO adducts where, upon the formation of an adduct, the isomer shift decreases, which is indicative of oxidation of the iron.

In summary, the in situ SERS spectrum of the nitrosyl adduct of Hm adsorbed on roughened Ag electrodes recorded with Q band excitation failed to reveal features attributable to the modes involving NO. In turn, NO binding to the macrocycle brings about a significant decrease in the intensity of the ν_{30} mode associated with the ring N-C_a group that is vicinal to the Fe center, providing a good marker for monitoring the dynamics of formation and subsequent electrochemical reduction of the NO adduct, which is now in progress in our laboratory.

References and Notes

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