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Evidence of electron-transfer in the SERS spectra of a single iron-protoporphyrin IX molecule

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Abstract

Surface enhanced Raman spectroscopy is applied to study single molecule iron-protoporphyrin IX, adsorbed on silver colloidal surfaces. If on one hand, drastic, random intensity and frequency fluctuations are observed in the Raman lines, on the other, a time dependent switching between Fe^{3+} and Fe^{2+} states is evidenced. Such a charge transfer, due to ballistic electrons oscillating across the molecule and the metal surface, could provide some insight on both the surface enhanced Raman mechanisms and the interfacial electron transfer in hybrid systems.
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1. Introduction

Spectroscopic signals from single molecules reveal drastic fluctuations whose analysis may provide information usually obscured in bulk measurements due to ensemble averaging [1–3]. The optical properties of single molecules, depending very sensitively on their interaction with the surrounding environment, might be used to locally monitor specific processes at molecular level [4]. Among other techniques, surface enhanced Raman spectroscopy (SERS) allows one to reach single molecule detection [5,6]. A drastic increase, up to 10^{14}, of the Raman cross section can be achieved by adsorbing molecules onto nanometer-sized metallic particles [7,8]. SERS signals from single molecules exhibit abrupt intensity and frequency changes, or deep spectral jumps, whose presence, still unclarified, cannot be simply attributed to a deviation from the ensemble average [9–14]. The investigation of the phenomena occurring under laser illumination could, moreover, help to understand the electron transfer at the interface between molecules and electrodes in hybrid nano-devices [4].

SERS is assumed to originate from two cooperating mechanisms: the electromagnetic enhancement (EM) effect associated with an enhanced local field caused by surface plasmon resonance, and the so-called chemical enhancement involving a bonding interaction between the molecule and the metal surface [7,8]. The EM effect depends on the microscopic shape of the metallic surface and has been shown to decrease progressively with the distance from the surface [7]. The chemical, or charge transfer (CT) mechanism, generally provides a smaller contribution to the total enhancement and is currently attributed to photon-driven CT between the molecular electronic states and the metal surface [8].

Iron-Protoporphyrin IX (FePP) constitutes the prosthetic group of several metallo-proteins, such as hemoglobin, myoglobin and cytochromes, in which it plays a key role, being also involved in electron transfer processes. FePP has been proposed also to be a suitable, robust element for assembling bioelectronic and photonic nano-devices [15–17]. Spectroscopic detection down to the level of single molecule of FePP and FePP-containing proteins has been successfully achieved by exploiting

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the SERS effect [9–14]. In the case of single FePP molecules, the peculiar fluctuations in their SERS spectra have been interestingly put into relationship either to a positional entanglement of the molecule or to a temporal gating of particular vibrational modes [10].

Here, we revisit the spectral jumps in the SERS spectra of single FePP molecule in the attempt to single out a vibrational fingerprint of an electron transfer occurring between the molecule and the metal surface.

2. Materials and methods

Solutions of colloidal silver particles have been prepared by standard citrate reduction of $AgNO_3$ (Sigma) by following the procedure of Lee and Meisel [18] and a concentration of silver particles of about 10^{-11} M, corresponding to about 7×10^{12} particles per liter has been estimated. As activation agent, NaCl has been added to reach the final concentration of 0.25 mM (for more details see [9,10]).

Fe-protoporphyrin IX (Sigma) has been dissolved into a KOH solution 0.5 M at pH 12 at a concentration of 10^{-4} M. An aliquot of successive dilutions of this solution has been incubated with silver colloidal suspension for 5 h at room temperature to obtain a final concentration of 10^{-11} M with an approximate 1:1 ratio between molecules and colloidal particles.

A droplet (about 10 μl) of this solutions has been deposited onto a glass slide, of area 15×15 mm^2, coated with polymerized 3-aminopropyltriethoxysilane (APES, Sigma) (see also [10]).

SERS spectra from FePP have been collected with a confocal Jobin–Yvon Labram equipment using the 514.5 nm radiation line of an Argon laser. The illumination and back-scattering collection system consists of a confocal microscope coupled to a single grating spectrometer (300 mm focal length spectrograph with a 1800 grooves/mm grating) and a liquid nitrogen cooled CCD detector. The CCD detector has, at the wavelength of 514.5 nm, a quantum efficiency of about 0.92 and it produces one count per two collected photons. The microscope objective is 100× with NA = 0.9, producing a laser spot size of about 1 μm in diameter. The spectral resolution is lower than 5 cm^{-1}. The laser power has been varied from 0.1 to 8 mW, resulting in to a range of 0.01–0.8 mW for the power impinging on the colloidal sample.

The scanning area containing immobilized Ag colloids incubated with FePP at 10^{-11} M alternatively reveals sites where only noise, at the CCD detector level is registered, and a few spots characterized by brightly emitting particles. SERS spectra arising from sites containing possible contaminations were discarded by preliminarly analyzing their spectral features. The number of discarded sites has been observed to decrease with

the power; such a behaviour finding a correspondence with that recently found for rhodamine [19].

3. Results and discussion

Fig. 1 shows a sequence of SERS spectra, recorded with 1 s of integration time and excited at a wavelength of 514.5 nm, from a typical bright site of immobilized Ag colloids incubated with FePP at 10^{-11} M. The spectra exhibit, similarly to what previously observed with a less sensitive CCD [10], a great spectral and intensity fluctuations. Lines appear and disappear suddenly and the relative peak intensity, as well as the frequency of the lines, drastically change in time; such a behaviour generally representing a hallmark for single molecule detection [9–14].

We have performed a detailed statistical analysis of both the intensity and frequency of the vibrational modes appearing in the spectra series.

Histogram in Fig. 2a shows the occurrence of the vibrational modes sampled in a sequence of 600 SERS spectra; each one recorded with 1 s of integration time. The resulting distribution reveals different

Fig. 1. Selected SERS spectra as obtained with 1 s of integration time, from a bright site of immobilized silver colloidal solution incubated with FePP at a concentration of 10^{-11}. The laser exciting wavelength is 514.5 nm. The microscope objective is a 100× with NA = 0.9 producing a laser spot size of about 1 μm in diameter.

Fig. 2. (a) Statistical analysis of the peaks appearance on 600 SERS spectra in sequence from a bright site of a dry sample constituted by silver colloidal solution, incubated with FePP at a concentration of 10^{-11} M (FePP to Ag particle ratio of 1:1); each spectrum was obtained with 1 s of integration time and separated 1.4 s from the previous and successive ones. (b) Collection of all the 600 SERS spectra. (c) Raman spectrum from a dry drop of FePP (10^{-4} M in alkaline aqueous medium) recorded with an integration time of 30 s. The marker is located at $\nu_4 = 1363$ cm^{-1}.

accumulations and, in addition, the peak positions appear be substantially coincident with those found when several single molecule spectra are summed up (see Fig. 2b).

Furthermore, the overall spectral features emerging in Figs. 2a and b are closely reminiscent of those observed from a droplet of FePP solution (10^{-4} M) dried on the measurement glass (see Fig. 2c). The Raman spectrum of FePP, as excited at 514.5 nm arises from vibrational modes strongly coupled to the tetrapyrrole $\pi \rightarrow \pi^*$ optical transition in the pre-resonant Q band (see [20] for a complete attribution of the bands). The finding that the spectral features obtained by summing several 1s spectra or, equivalently, from a SERS spectrum with a long integration time, were similar to the features of a great ensemble of FePP molecule, is indicative of a substantial ergodicity of the system. On the other hand, the recovering of the FePP spectral features in the SERS spectrum of Fig. 2b provides a confirmation for the fact that no degradation has taken place in our sample during the measurements.

We have restricted our attention to the spectral region of the ν_4 band, corresponding to the pyrrole half-ring vibration: this band is located at 1363 cm^{-1} for bulk FePP (see Fig. 2c), and marks a ferrous state for iron, while such a band is expected at a higher shift (1375 cm^{-1}) for a ferric state. This band appears somewhat broadened, toward slightly higher frequency modes, in spectrum of Fig. 2b which was derived by summing all the spectra in the sequence; such a finding suggesting a contribution from molecules in different oxidation

states. To investigate changes in the oxidation state of FePP iron, the behaviour of lines at 1363 cm^{-1} (indicative of a ferrous state) and 1375 cm^{-1} (indicative of a ferric state) has been followed in the spectra sequence.

Fig. 3 shows the occurrence of the lines 1363 cm^{-1} (squares) and 1375 cm^{-1} (circles) according to the spectrum number. Notably, the two peaks are alternatively observed in the sequence number for the largest part of the spectra; a few cases showing a simultaneous detection of both lines being also detected (triangles in Fig. 3).

These results indicate that changes in the oxidation state of iron occur during the 1 s measurements. This is consistent with the hypothesis that light excitation should involve a non-radiative CT mechanism between FePP single molecule and the silver surface. Actually, ballistic electrons from the silver surface have been suggested to jump non-radiatively towards the FePP molecule and backwards [21].

To get further information, an analysis of the intensity trend of the 1363 and 1375 cm^{-1}, lines has been performed as function of the spectrum number. Moreover, to get rid of the noise into the fluctuations we have also analyzed the intensity trend at 1800 cm^{-1}, where no Raman signal is observed (see Fig. 4). The intensity at the latter frequency is taken, therefore, as an estimation of the continuum signal underlying the SERS response; the background signal as due to fluorescence, commonly affecting Raman spectra, is expected to be quenched in the proximity of a metal surface [21].

We would like to remark that a high Raman intensity, at a given frequency in a spectrum, does not necessarily imply the occurrence of a peak at that frequency. Actually, a variety of factors may independently affect the intensity and the peak appearance. The intensity is mainly related to the enhancement factor, while the

Fig. 3. Appearance of Raman peaks, over the background, as a function of the spectrum number, on 600 SERS spectra in sequence from silver colloids incubated with FePP 10^{-11} M, at 1363 cm^{-1} (squares), at 1375 cm^{-1} (circles) and at both 1363 and 1375 cm^{-1} (triangles).

Fig. 4. Raman intensity, as a function of the spectrum number, from the top to the bottom at 1363, 1375, and 1800 cm^{-1} for a sequence of 600 SERS spectra, with 1 s of integration time, and lasting 1.4 s between two successive spectra, from silver colloids incubated with FePP 10^{-11} M. The two ρ values indicate the correlation, at 0 time delay, of the Raman intensity, at the analyzed two frequency, with that of the underlying continuum (see Eq. (1)).

appearance of a peak, at a given frequency, directly reflect the dynamical response of the molecule, likely dependent on the external environment.

As expected, from being in the single molecule regime, drastic fluctuations in the intensity are revealed in all the cases. It is worth of note that the spectrum numbers at which both the 1363 and 1375 cm^{-1} peaks are detected, correspond in most cases to those spectra characterized by a high background intensity (see markers in Fig. 4).

At visual inspection, the intensity trend of the lines at 1363 and 1375 cm^{-1} appears very similar; in addition, such a trend is closely correlated to that of the underlying continuum. Such an aspect has been quantitatively addressed by evaluating the cross-correlation ρ between the Raman intensities at 1363 and 1375 cm^{-1} and that of the continuum intensity [9,10]; ρ being expressed by

$$\rho = \frac{\sum_i [I_i(v) - \bar{I}(v)][I_i(v_{\text{background}}) - \bar{I}(v_{\text{background}})]}{\left\{\sum_i [I_i(v) - \bar{I}(v)] \sum_i [I_i(v_{\text{background}}) - \bar{I}(v_{\text{background}})]\right\}^{1/2}},$$

(1)

where $I_i(v)$ is the Raman intensities, detected at the wavelength v for the spectrum i; $I_i(v_{\text{background}})$ is the

Raman intensity, detected at the 1800 cm^{-1}; $\bar{I}(v)$ and $\bar{I}(v_{\text{background}})$ being the intensities averaged over the spectra series at the frequency v and 1800 cm^{-1}, respectively.

Correlation values higher than 0.87 (see Fig. 4) indicate that a relevant contribution to the intensity fluctuations of the 1363 and 1375 cm^{-1} lines comes from the continuum underneath the SERS lines in agreement with what previously observed [9,10]. Such a result is consistent with the hypothesis that the fluctuating broad band continuum arises from a byproduct of the non-radiative CT process between the metal surface and the molecule [14]. Indeed, the higher is the non-radiative CT transfer, the stronger is the background intensity, and then the Raman enhancement. Accordingly, a higher probability to have changes in the oxidation state of the FePP molecule is expected. On such a ground, by considering that 200–800 photons are collected in our single molecule experiments [9,10], it can be conceived that, sometimes, both the oxidation states could be detected during the collection time of one second.

Even if the possibility to reveal more than one molecule with different oxidation states in the measurements cannot be ruled out, from a quantitative point of view, our data appear to be more consistent with the presence of a single molecule in the scattering area.

The detected changes in the iron oxidation state of FePP are the result of a visible light-induced process. Indeed, a more efficient non-radiative CT transfer would be coupled to a higher background intensity and then to a more efficient Raman enhancement.

In general, the photochemical event involves photon driven transfer of an electron from a metal state to an unoccupied molecular orbital of the adsorbate. Actually, in a molecule-metal system, the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) of the adsorbed molecule are expected to be symmetrically located in energy with respect to the Fermi level of the metal which acts as an initial or final state in the resonance Raman process [8,21,22]. The HOMO and LUMO levels of FePP have been evaluated to be located at −0.48 and 0.40 eV, respectively [23]; while the energy Fermi level for silver is 5.49 eV. Therefore, the related positions among the HOMO, LUMO levels and the Fermi energy of the FePP–silver system do not properly match the above mentioned conditions for the CT mechanism to occur; a shift of some or all these levels being then required to match the CT conditions. We mention that a change in the Fermi energy level may occur as induced by doping a metal surface with very small amount of either electropositive or electronegative elements [8]. On the other hand, a shift of HOMO and LUMO levels, as well a variation in the HOME–LUMO gap, can be induced when a molecule is adsorbed on to silver metal [24,25].

Finally, it should be mentioned that molecular oxygen might play some role in the CT mechanism acting as an intermediate state in the transfer process between the molecule and the metal surface, as recently suggested [25].

4. Conclusions

Consecutive Raman SERS spectra, at level of single FePP molecule adsorbed on silver colloids, indicate light-induced changes in the oxidation state of iron. Such a phenomenon, reflecting an electron transfer taking place between the molecule and the metal surface, could provide some insight on both the understanding of the SERS mechanism and the interfacial electron transfer process occurring in hybrid systems as driven by visible photons.

Acknowledgements

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