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Surface-Enhanced Resonance Raman Scattering of Porphyrins on Gold Nanoparticles Attached to Silanized Glass Plates

Abstract: Surface-enhanced resonance Raman scattering (SERRS) spectra of cationic 5,10,15,20-tetrakis(1-methyl-4-pyridyl) porphyrin (TMPyP) and anionic 5,10,15,20-tetrakis(4-sulfonatophenyl) porphyrin (TSPP) were measured from gold surfaces prepared by attaching citrate-reduced colloidal nanoparticles to glass slides silanized by 3-aminopropyltrimethoxysilane. SERRS spectra of both porphyrins obtained in a large concentration range (1×10^{-4} to 1×10^{-7} M) of primary solution do not show any sign of porphyrin metalation or perturbation of its native structure. Optimal adsorption time (15–20 min) and covering concentration limit (lower than 1×10^{-5} M) of porphyrins have been estimated from the concentration and soaking time dependences of SERRS spectra. © 2006 Wiley Periodicals, Inc. *Biopolymers* 82: 390–393, 2006

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INTRODUCTION

Porphyrins are important biomolecules and their derivatives are or can potentially be applied to photodynamic therapy of cancer, antiviral treatments, molecular biology, specific sensing of DNA sequences, selective cleavage of nucleic acids, etc.

Porphyrins have been extensively studied by using surface-enhanced resonance Raman scattering (SERRS) spectroscopy, employing silver surfaces including silver colloids.¹ In the case of free-base porphyrins, metalation process (incorporation of a metal ion from the surface into the porphyrin core) has been observed. The metalation substantially decreases reproducibility of porphyrin SERRS spectra and even makes obtaining SERRS spectra of unperturbed free-

base porphyrin impossible. Although considerable attention has been paid to discovering how to prevent free-base porphyrins from metalation (e.g., by using an efficient molecular spacer), it seems to be extremely difficult in some cases.²

In SERS applications, metal colloidal nanoparticles are the most frequently used substrates due to their cheap and simple preparation but a serious drawback for their analytical applications is their chemical instability and irreproducibility of spectral measurements. A novel method of solid SERS-active surface preparation, metal colloidal nanoparticles attached to glass slides with self-assembled monolayer of organosilanes, has recently been developed.^{3–6} This method is in principle suitable for preparation of stable and inexpensive SERS-active substrates with reproducible signals.

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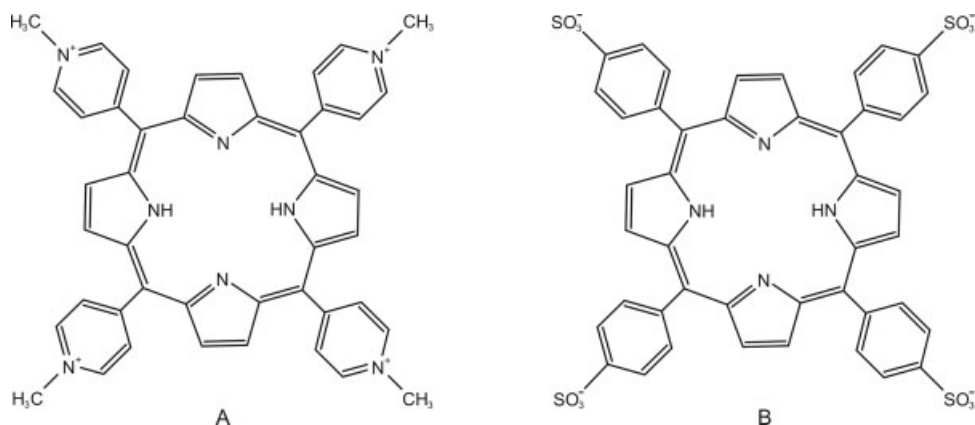


FIGURE 1 Chemical structure of TMPyP (A) and TSPP (B).

This contribution deals with two kinds of free-base porphyrins, cationic 5,10,15,20-tetrakis (1-methyl-4-pyridyl) porphyrin (TMPyP) and anionic 5,10,15,20-tetrakis(4-sulfonatophenyl) porphyrin (TSPP) (see Figure 1 for chemical structures), on a gold surface prepared by attaching citrate-reduced colloidal nanoparticles to glass slides silanized by 3-aminopropyltrimethoxysilane. Dependence of porphyrin SERRS spectra on soaking time and porphyrin concentration were measured and discussed.

EXPERIMENTAL

Deionized water of a resistance of 18 M Ω was used for all preparations. H₂SO₄ (96%), H₂O₂ (30%), HCl (35%), and HNO₃ (65%) were obtained from Lachema, BRNO, Czech Republic. HAuCl₄, sodium citrate, methanol (99.8%), 3-aminopropyltrimethoxysilane (APTMS, 97%), TMPyP, and TSPP were purchased from Sigma-Aldrich (S.P.D., Prague, Czech Republic). All glassware was cleaned using "piranha" solution (4 parts H₂SO₄, 1 part H₂O₂) to remove organics and then aqua regia (3 parts HCl, 1 part HNO₃) for removal of metals.

Citrate-reduced (c.-r.) colloid was prepared by reduction of HAuCl₄ by sodium citrate. 250 mL of 1 mM solution of HAuCl₄ was brought to a boil and then 25 mL of 38.8 mM solution of sodium citrate was added. Boiling was continued for 15 min.

SERS substrates were prepared according to reference 5 for more details see reference 7. Clean glass slides (1 \times 2 cm² strips) were derivatized in 10% solution of organosilane in methanol for 30 min. After silanization, substrates were rinsed several times with methanol and then with water to remove any physisorbed organosilane, which could cause gold colloidal particle aggregation in the colloidal suspension during the next step. Each silanized glass plate was dipped in a vertical position (thus the gold surface was formed on both sides of the glass slide) into a tube containing 1 mL of the colloidal suspension for 3–4 h. They then

were rinsed with water. Afterward the substrates were left to dry at 100°C.

Dependence of the SERRS spectra on the soaking time were measured as follows: glass substrate was placed for an appropriate period in contact with the porphyrin solution and then was rinsed by deionized water and SERRS spectrum was measured. The glass substrate was then placed again in the same porphyrin solution for additional time, rinsed by deionized water, and the SERRS spectrum then was measured. The soaking–rinsing–measuring cycles were repeated gradually and the total time of soaking was counted. The concentration dependence of the SERRS spectra was measured in the same way, but the soaking time was fixed and the porphyrin concentration was gradually increased.

SERRS spectra were recorded from the substrate at room temperature with a Raman spectrometer using the 514.5 nm excitation line of an Ar⁺ laser ILA (Carl Zeiss,

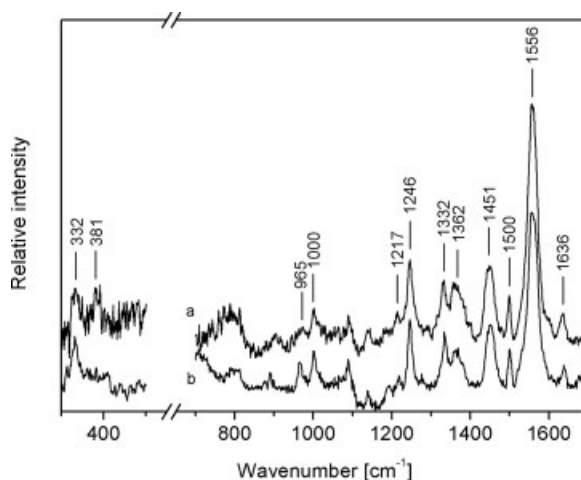


FIGURE 2 SERRS spectrum of 1 μ M TMPyP measured from Au surfaces (a) and RRS spectrum of 1 mM TMPyP measured from a dried drop on a glass slide (b). The baseline was corrected and the Raman signal of glass subtracted.

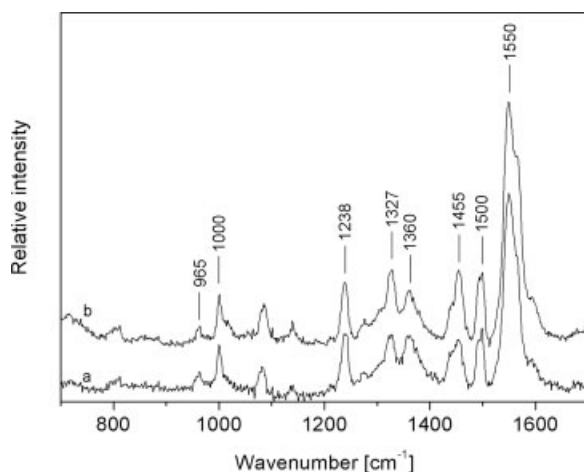


FIGURE 3 Same as Figure 2 legend for TSPP.

Jena, Germany, power ~ 150 mW at the sample) in reflection geometry. Scattered radiation was collected at 90° relative to the excitation beam and detected with a nitrogen-cooled CCD.

RESULTS AND DISCUSSION

Figures 2 and 3 show SERRS spectra of $1 \mu\text{M}$ porphyrins measured from Au substrate (a) and resonance Raman spectra (RRS) of 1 mM porphyrins measured from a dried drop on a pure glass slide (b). Since no Raman signal was detected from a drop of the same porphyrin solution on a glass surface without gold nanoparticles, it is certain that a pure SERRS spectrum of porphyrins was obtained. SERRS spectra of both porphyrins are similar showing typical bands corresponding to vibrations of the porphyrin macrocycle at ~ 332 , 970 , 1000 , 1215 (TMPyP), 1238

(TSPP), 1246 (TMPyP), 1332 , 1362 , 1453 , 1500 , 1555 , and 1636 (TMPyP) cm^{-1} . Comparison of our SERRS spectra with the well-known free-base and metalation markers¹ as well as with RRS spectra of 1 mM porphyrin (see Figures 2 and 3, spectra b) clearly proves that for both porphyrins we obtained SERRS spectra of their free-base forms. It means that the gold surface, in contrast to silver surfaces,¹ does not change the chemical structure of the free-base porphyrins. It is therefore a very suitable substrate for SERRS spectroscopy of free-base porphyrins. SERRS spectra of TMPyP and TSPP taken from different spots of the surface and spectral series obtained from a set of various plates have proved homogeneity of the gold surface and reproducibility of SERRS spectra.⁷

Time evolution of the porphyrin adsorption was monitored via SERRS spectra obtained for different soaking times (from 1 to 60 min). Factor analysis (FA)¹ of the spectral sets shows that the factor dimension is just one, evidencing that no porphyrin metalation nor other changes of its form occur even for long soaking times or various porphyrin concentrations. The soaking time dependences obtained from FA results of $1 \mu\text{M}$ TMPyP and TSPP spectra are shown on the left side of Figure 4. The soaking time of 15–20 min seems to be optimal for both porphyrins showing maximal SERRS signal. On the other hand, while in the case of TMPyP the SERRS signal is nearly constant for longer times, it substantially decreases (less than 40%) in the case of TSPP.

Dependences of the SERRS spectra on starting concentrations of the porphyrin solution used for soaking were measured in 1×10^{-7} to $1 \times 10^{-4} \text{ M}$ range (for 20 min soaking time). After the FA treatment, the results shown on the right side of Figure 4 were obtained. For both porphyrins, the SERRS sig-

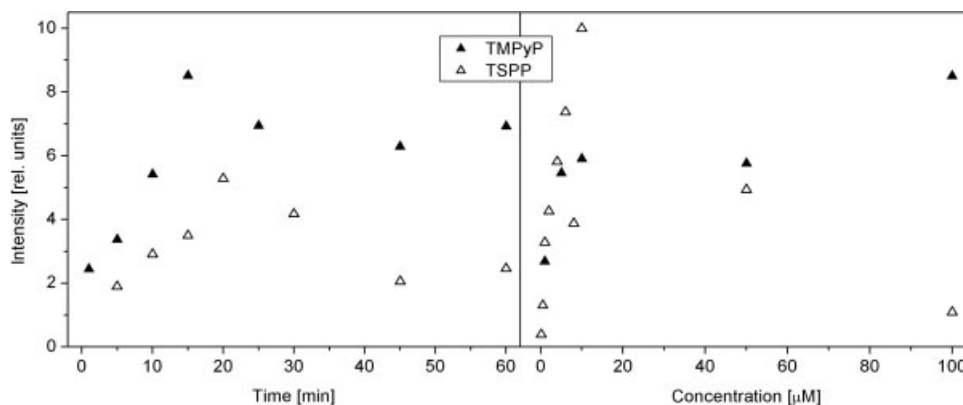


FIGURE 4 (Left) SERRS intensity of TMPyP and TSPP versus soaking time ($1 \mu\text{M}$ porphyrin concentration). (Right) SERRS intensity of TMPyP and TSPP versus concentration of employed porphyrin solutions (soaking time of 20 min).

nal increases rapidly up to $\sim 1 \times 10^{-5} M$ concentration. In case of 100% efficiency of the porphyrin adsorption, this level should correspond to a covering limit of the porphyrin molecules on the gold surface. Although we expect lower real porphyrin concentration at the surface (not possible to be determined from our data).

While the TMPyP shows typical saturation behavior of the concentration dependence, the SERRS signal of TSPP substantially decreases for higher concentrations (even down to 10% of maximal value). Similar behavior has been observed for various molecules (such as cyanide anions) when a monolayer coverage was exceeded and interpreted as a depolarization effect of neighboring molecules.⁸ Accordingly we interpret both the decrease of the TSPP SERRS signal at longer soaking times and at higher porphyrin concentrations as a depolarization effect of TSPP molecules. Absence of this effect for TMPyP may indicate different adsorption behavior very probably due to the different charge.

CONCLUSION

SERRS spectra of two porphyrin derivatives have been obtained in a large concentration range (1×10^{-4} to $1 \times 10^{-7} M$) using gold nanoparticles immobilized onto silanized glass plates as a SERS-active surface were found to be a unique way how to obtain SERRS spectra of free-base porphyrins including those exhibiting rapid metalation in Ag-based SERS-active systems (TMPyP).

An optimal adsorption time of 15–20 min, covering concentration lower than $1 \times 10^{-5} M$ and detection limit of about $1 \times 10^{-7} M$, was detected for both

TMPyP (cationic) and TSPP (anionic) porphyrins at these experimental conditions. In the case of TSPP, the SERRS signal substantially decreased for the longer soaking times or the higher porphyrin concentration than the optimal values.

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