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In-situ surface-enhanced Raman spectroscopic investigation of NH site of indole ring-terminated self-assembled monolayer on gold electrode

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Isotopic H / D exchange and hydrogen bonding interaction at N1H site of indole ring-terminated self-assembled monolayer (SAM) on gold electrode in neutral aqueous solutions was studied by surface-enhanced Raman spectroscopy (SERS). From an analysis of W17 mode (a mixture of benzene ring and N1H group motion) in electrolytes prepared from H_2O and D_2O solvent it was found that water accessibility to the indole N1H sites increases at –0.70 V electrode potential as compared with 0.30 V (vs Ag / AgCl, 3M KCl). Data on potential dependence of the W17 mode frequency suggested an increase in hydrogen bonding interaction strength at N1H indole site at sufficiently negative electrode potentials.

Key words: indole, tryptophan, gold, SAM, surface enhanced Raman spectroscopy

INTRODUCTION

An interaction of tryptophan side chains with other amino acid residues in proteins and enzymes plays an important role in controlling the folding process [1–3]. The indole ring of tryptophan is able to stabilize the tertiary structure by hydrogen bonding interaction through the N1H group (Scheme), π–π interaction with other aromatic residues, and cation–π interaction between the positively charged metal cation (Na^+, K^+) and delocalized π–electron cloud of the ring [2–5]. These complex interactions are difficult to study in real systems, therefore, a construction of well-defined model systems with properties variable in a controllable manner is highly needful. Self-assembled monolayers (SAMs) constructed by the adsorption of thiols at metal (Au or Ag) surfaces offer a possibility to create stable molecular structures suitable for application in fundamental studies of electron transfer processes [6, 7], ion-pairing at interfaces [8–12], interaction between the terminal functional group of the monolayer and solution species [7], and electrocatalytical reactions [11, 12].

Among the methods suitable for the structural characterization of the monolayer at molecular level, surface-enhanced Raman spectroscopy (SERS) seems to be particularly attractive because of the possibility to record *in-situ* vibrational spectrum of less than a monolayer of surface-confined species in a wide frequency region (100–3700 cm^{-1}), in an aqueous solution, and at controlled electrode potential [13–17]. Previously we have studied the state of the indole ring of tryptamine immobilized on polycrystalline gold electrode by means of electroreflectance spectroscopy, ellipsometry, and SERS techniques [18]. In that study, tryptamine was immobilized on the Au surface modified by the protein cross-linking Lomant's reagent [19]. The structure, surface coverage, and properties of such surface layer, in general, might be different as compared with SAM formed from bifuncional thiol molecules bearing an indole ring functional group. Recently, we have synthesized a new indole ring-terminated thiol, ω-mercaptooctyltryptamide (MOTA), and studied the structure of its SAM on Ag electrode by SERS [20]. We have shown that the frequency of W3 mode (pyrrole ring-stretching vibration localized primarily at the C2=C3 bond) is sensitive to the electrode potential, and blue shifts at more negative potential values. In addition, it was demonstrated that the hydrogen bonding interaction strength at N1H indole site increases in slightly alkaline solutions as compared with acidic conditions.

The present work is devoted to the *in-situ* SERS study of the hydrogen bonding interaction and isotopic H / D exchange at N1H site of SAM formed from MOTA compound at gold electrode.

EXPERIMENTAL

Materials

8-bromooctanoic acid (97%) and tryptamine (98%) was obtained from Aldrich and was used without additional purification. Sodium phosphate monobasic and sodium phosphate dibasic were ACS reagent grade (ACROS ORGANICS). Deuterium oxide was purchased from Sigma-Aldrich Chemie GmnH and used without further purification. Deionized and subsequently double-distilled water was used throughout the experiments. Some experiments were conducted in the Millipore purified (18.2 MΩ cm) water. MOTA compound was synthesized as described in [20].

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Raman measurements

Surface-enhanced Raman spectra were recorded using a 500 mm focal length, f/6.4 aperture ratio spectrograph (Acton Research Co., Model: SpectraPro-2500i) equipped with 600 lines/mm grating and a thermoelectrically cooled (–60 °C) CCD camera (Princeton Instruments, Model: Spec-10 : 256E). The cut-off filter (Semrock Inc.) was placed in front of the entrance slit of the spectrograph to eliminate Rayleigh scattering from the sample. The Raman measurements were carried out in 90° geometry. The 676.4 nm beam of a Kr-ion laser (Coherent, Model: Innova 90-K) was used as the excitation source. The incidence angle of the laser beam was 60° and the laser power at the sample was typically 10–30 mW. The Raman frequencies were calibrated using the toluene spectrum. The integration time was 1 second. Each spectrum was recorded by the accumulation of 30 scans. In order to increase the signal-to-noise ratio, 5 spectra were averaged. In order to reduce photo- and thermoeffects, the cell together with the electrodes was moved linearly in respect of the laser beam with a rate of about 20 mm/s [21].

Near-infrared SERS measurements were carried out in 180° geometry with an FT-Raman spectrometer (Perkin-Elmer, Model Spectrum GX) equipped with an InGaAS detector operating at room temperature. The excitation was provided by an air-cooled diode-pumped Nd-YAG laser with an emission wavelength of 1064 nm. The laser beam was focused to a spot of area ~1 mm^2, and the laser power at the sample was set to 300 mW. The experiments were carried out in 180° geometry. The spectral resolution was set at 4 cm^{-1} and the wavenumber increment per data point was 1 cm^{-1}. All of the spectra were acquired by 300 scans. None of the spectra presented was smoothed.

SERS measurements were performed in a cylindrical closed three-electrode cell made of glass. A polycrystalline rod of Au (99.99% grade) pressed into a Teflon sleeve served as the working electrode. The counter electrode was a Pt wire. The potential of the working electrode was measured vs Ag / AgCl, 3M KCl reference electrode. All the potentials are given vs this electrode. Before each experiment, the working electrode was polished on alumina (Struers, Denmark) slurry of 0.20 and, subsequently, of 0.05 μm and then sonicated for 3 min in a 1 : 1 mixture of water and ethanol, washed with water and electrochemically cleaned by keeping the electrode potential at –1.0 V for 1 min in 0.1 M NaCl solution. After these procedures, the electrode was electrochemically roughened by 50-fold scanning the electrode potential from –0.30 to 1.30 V (sweep rate 500 mV/s) with holds of 90 s at the negative potential and 2 s at the positive one, like it had been reported previously [10, 22]. After the roughening, the electrode was immersed into the ethanolic 1 mM MOTA solution for 24 hours. Subsequently, the electrode was rinsed with ethanol and water and transferred with a protective drop of water to the spectroelectrochemical cell containing the working solutions. Electrochemical control was accomplished by using a PI-50-1 model potentiostat, arranged with a PR-8 model programmer.

RESULTS AND DISCUSSION

Because of the extended aromatic ring system, the indole ring of tryptophan amino acid residue is a relatively hydrophobic group and plays an important role in the interaction of biomolecules with lipid acyl chains [23]. However, indole ring also contains a hydrophilic center, N1H group (Scheme), able to participate in the hydrogen bonding interaction. For indole ring-terminated SAM, the accessibility of N1H group to water molecules might be restricted because of the compact arrangement of bulky aromatic groups. In this work, we have used SERS technique to get a better insight of the hydrogen bonding interaction of indole ring-terminated monolayer. Fig. 1 shows SERS spectra of SAM of MOTA compound on the gold electrode in aqueous solutions prepared with H_2O or D_2O solvent. The dominant bands in the spectra are associated with the vibrations of the indole ring [20]. Table summarizes the assignments of the peaks based on previous reports [24–27]. It should be noted that MOTA monolayer spectrum on the Au electrode differs slightly as compared with Ag at the same electrode potential. Interestingly, the W3 peak position for the monolayer on the Au surface shifts slightly to lower wavenumbers (2–3 cm^{-1}) as compared with Ag electrode (676.4 nm excitation), indicating subtle changes in the torsion angle around the Cβ–C3 bond [25]. We were not able to distinguish any features due to the vibrations of amide linkage, presumably because of relatively low Raman cross section for amide modes as compared with aromatic ring bands. Two bands located at 1078 and near 1445 cm^{-1} were attributed to C–C stretching and CH_2 scissoring bending vibrations of alkyl chain, respectively [28]. In a region of low frequency (not shown) we were able to identify Au–S stretching vibration at 280 cm^{-1} directly indicating the formation of gold–sulfur bond. The other band, presumably, associated with the motion of gold and sulfur atoms was detected near 180 cm^{-1}.

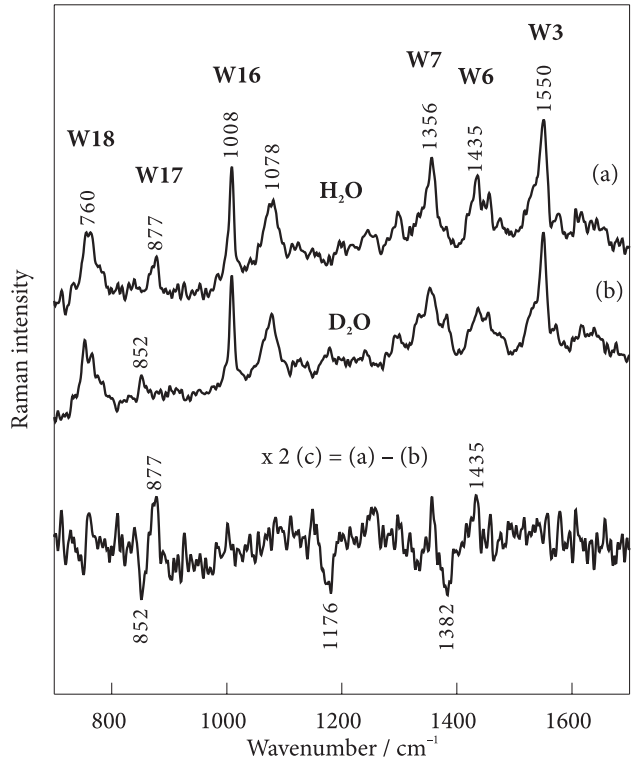
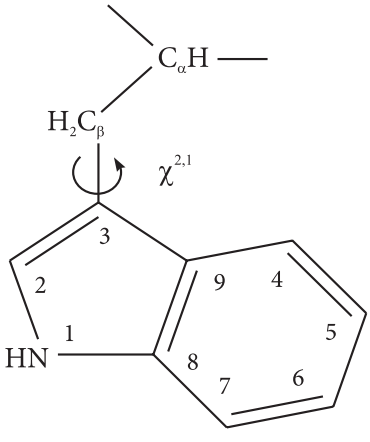


Fig. 1. SERS spectra of indole-ring terminated SAM on gold electrode at 0.30 V (vs Ag / AgCl, 3 M KCl) observed in 0.1 M Na_2SO_4 solution containing 0.01 M sodium phosphate buffer (pH 7.0) prepared with H_2O (*a*) or D_2O (*b*) solvent. Difference spectrum (*c*) is also shown. Excitation wavelength is 1064 nm. The spectra were obtained after the excursion to –0.70 V for 20 min

Let us consider the parameters of indole ring bands in H_2O and D_2O solutions. SERS technique is able to provide information on the accessibility of the indole ring to solution water molecules, because the modes coupled with the motion of N1H group (Scheme) must shift in frequency. Several changes in band positions due to the solvent H_2O exchange to D_2O are clearly visible in the difference spectrum (Fig. 1c). The derivative-like feature in the vicinity of W17 mode shows a decrease in the peak frequency from 877 cm^{-1} in H_2O solution to 852 cm^{-1} in D_2O. This is because W17 mode involves the deformation vibration of N1H group, and therefore, is one of the hydrogen-bonding interaction markers of the indole ring [25, 26]. The second feature, clearly sensitive to H / D exchange at the nitrogen atom of the indole ring, is the peak located at 1435 cm^{-1} (W6), which in D_2O solution shifts to 1382 cm^{-1}. It should be noted that isotopic changes for W6 mode is not obvious in the original spectra of the monolayers, because of the overlap with the vibrations of alkyl chains of MOPA compound. However, the isotopic shift is clearly visible in the difference spectrum, which selectively probes the spectral changes associated only with H / D exchange at the nitrogen atom of the indole ring. The W6 mode involves stretching vibration of the pyrrole ring N1–C2=C3 moiety and N1H deformation motion coupled with CH deformation of the benzene ring (Table) [25, 26]. It should be noted that the origin of the negative feature in the difference spectrum at 1170 cm^{-1} remains unclear. The discussed spectral changes unambiguously indicate that under the studied experimental conditions, H / D exchange takes place at the ring nitrogen atom of SAM on the Au electrode, and therefore, the water molecules are able to access the indole functional group.



Scheme. Structure and atom numbering scheme for tryptophan derivatives

Preliminary studies have indicated that the rate of H / D exchange process is potential dependent. Fig. 2 demonstrates the influence of the electrode potential on the H / D exchange process at the ring nitrogen atom site. We have monitored spectral changes in the parameters of the hydrogen bonding marker mode W17, which shifts from 876 cm^{-1} to 852 cm^{-1} upon a transformation of N1H to N1D, respectively (Table). After the immersion of the Au electrode modified by SAM into D_2O-based electrolyte at 0.30 V (Fig. 2a) only a part of N1H sites transforms to N1D as it is evident from near-equal intensities of 852 and 877 cm^{-1} components of W17 mode. A little decrease in the intensity of the 877 cm^{-1} component was detected after 20 min at 0.30 V (Fig. 2b), indicating that the majority of the labile hydrogens of N1H site had already been exchanged by deuteratons. The remaining indole rings seems to be buried at the interface,

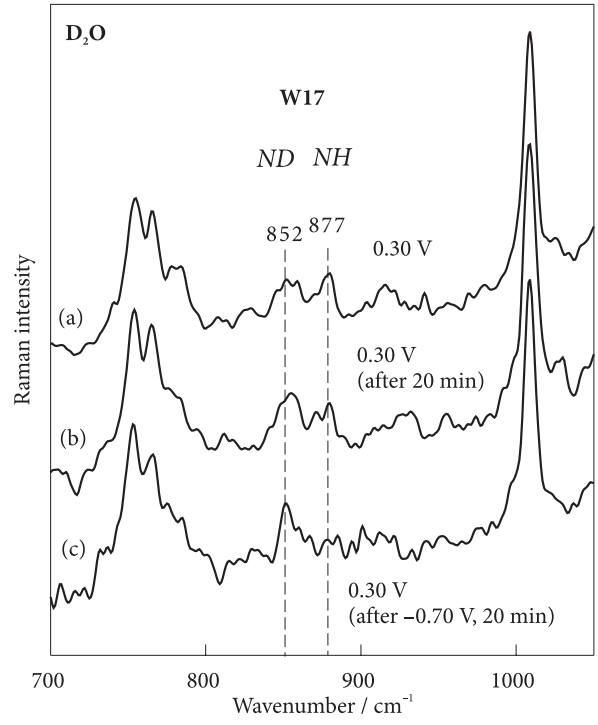


Fig. 2. SERS monitoring of H / D exchange process at N1H site of indole ring-terminated SAM on Au electrode. Spectra were obtained (*a*) immediately after immersion of electrode in 0.1 M Na_2SO_4 solution containing 0.01 M sodium phosphate buffer (pH 7.0) prepared with D_2O solvent at 0.30 V, (*b*) after 20 min at 0.30 V, and (*c*) after excursion of electrode potential to –0.70 V for 20 min and return back to 0.30 V. Excitation wavelength is 1064 nm

Table. **Frequencies, deuteration shifts (Δ) and assignments of selected bands of indole ring-terminated SAM on Au electrode in 0.1 M Na_2SO_4 solution containing 0.01 M phosphate buffer prepared with H_2O and D_2O solvent at 0.30 V electrode potential**

Frequency[a] (cm^{-1})		Δ[b] (cm^{-1})	Mode	Assignment[c]
H_2O	D_2O			
1550	1550	0	W3	Pyrrole ν(C2 = C3)
1435	1382	–53	W6	Pyrrole [ν(N1–C2=C3) + δ(NH)] + benzene δ(CH)
1356	1353	–3	W7	Indole ν(N1–C8), Fermi resonance
1008	1008	0	W16	Benzene ring breathing
876	852	–24	W17	Benzene (12 mode) + N1H motion
760	758	–2	W18	Indole ring breathing

[a] From 1064 nm-excited SERS spectra; [b] $\Delta = \nu(D_2O) - \nu(H_2O)$; [c] Based on references [24–27]. Abbreviations: ν – stretching vibration; δ – deformation vibration.

and D_2O molecules hardly approach the N1H sites at 0.30 V. It was found that H/D exchange rate considerably depends on the electrode potential. Spectrum (*c*) of Fig. 2 clearly shows a complete disappearance of the W17 mode component due to the N1H groups at 877 cm^{-1} after holding the electrode potential at –0.70 V for 20 min. The presented spectral data demonstrate potential-induced conformational changes in the monolayer at relatively negative electrode potentials resulting in an increase in water accessibility to the indole N1H sites.

The indole ring forms a hydrogen bond at the N1H moiety as a proton donor. Thus, the vibrational modes coupled with N1H vibrations may serve as hydrogen bonding marker bands [25–27]. We have found that the frequency of the W17 mode slightly depends on the electrode potential (Fig. 3). Given the sensitivity of the W17 mode frequency to hydrogen bonding (lower frequency reflects stronger bonding) [25–27], the presented data suggest an increase in the hydrogen bonding interaction strength at N1H indole site at sufficiently negative electrode potentials.

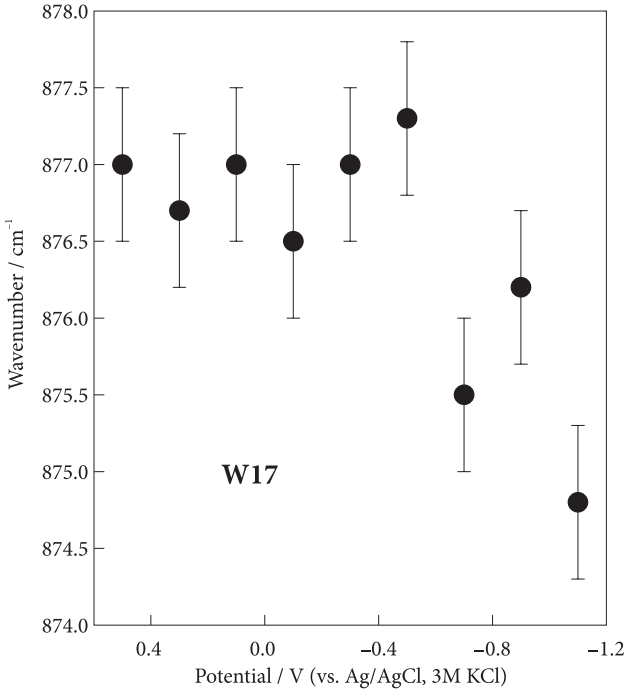


Fig. 3. Potential dependence of W17 peak position in SERS spectra of indole ring-terminated SAM on Au in 0.1 M Na_2SO_4 solution containing 0.01 M sodium phosphate buffer (pH 7.0). Excitation wavelength is 1064 nm

CONCLUSIONS

We have studied the structure of indole ring-terminated self-assembled monolayer on gold electrode in neutral aqueous solutions by using surface-enhanced Raman spectroscopy (SERS). The experiments performed in H_2O- and D_2O-based solutions revealed a potential dependence of N1H / N1D exchange process. Fast isotopic exchange was detected at relatively negative (–0.70 V as compared with 0.30 V vs Ag / AgCl, 3M KCl) electrode potentials. The W6 and W17 modes were found to be most sensitive to the isotopic exchange at the indole ring nitrogen atom. These modes shifted from 1435 (W6) and 877 cm^{-1} (W17) in H_2O solution to 1382 and 852 cm^{-1} in D_2O electrolyte, respectively. The analysis of the potential dependence of the W17 mode revealed an increase in the hydrogen bonding interaction strength at N1H indole site at sufficiently negative electrode potentials.

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SAVITVARKIO MONOSLUOKSNIO ANT AUKSO ELEKTRODO INDOLO ŽIEDO NH GRUPĖS IN SITU TYRIMAS SUSTIPRINTOS PAVIRŠIUMI RAMANO SPEKTROSKOPIJOS METODU

S a n t r a u k a

Sustiprintos paviršiumi Ramano spektroskopijos metodu neutraliuose vandeniniuose tirpaluose buvo tirti savitvarkio monosluoksnio ant aukso elektrodo indolo žiedo N1H grupės izotopiniai H / D mainai ir vandenilinio ryšio sąveika. Analizuojant W17 smailę (benzeno žiedo ir N1H grupės mišrus virpesys) H_2O ir D_2O tirpaluose, nustatyta, kad, esant -0,70 V potencialui, vanduo lengviau pasiekia N1H grupę, lyginant su 0,30 V (atž. Ag / AgCl, 3M KCl) reikšme. W17 smailės padėties priklausomybės nuo potencialo duomenys rodo, kad N1H grupės vandenilinio ryšio sąveika sustiprėja, esant pakankamai neigiamiems elektrodo potencialams.