1 ФОТОНИКА И ОПТОИНФОРМАТИКА PHOTONICS AND OPTOINFORMATICS

UDC 543.424.2, 538.97

SERS OF BACTERIORHODOPSIN WITH OUT-DIFFUSED SILVER NANOISLANDS

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Abstract. We present the studies on surface-enhanced Raman spectroscopy (SERS) of bacteriorhodopsin in purple membranes using self-assembled silver nanoisland films for Raman signal enhancement. These metal island films were fabricated on soda-lime glass slides subjected to silver-sodium ion exchange in molten $Ag_{0.05}Na_{0.95}NO_3$ at the temperature of 325°C for 20 minutes and subsequent treatment in hydrogen atmosphere at the temperature of 250°C for 10 minutes. The films typically consisted of 20–30 nm closely placed nanoislands. Being tested as SERS substrates for rhodamine 6G the nanoisland films gave the possibility to observe respective characteristic Raman lines from a dried drop of rhodamine 6G dissolved in water in the concentration of 10^{-6} M. Similarly fabricated substrates were used to obtain SERS spectra of bacteriorhodopsin in purple membranes dispersed in water, and Raman peaks at 1000–1020 cm^{-1}, 1150–1220 cm^{-1} and 1530–1570cm^{-1} were resolved. The substrates made it possible to register characteristic Raman peaks only for an order of magnitude lower concentration of bacteriorhodopsin in contrast to the virgin glass substrate, that is the enhancement of Raman signal was considerably less than for rhodomin 6G. This is supposed to be due to bacteriophodopsin molecules packing in patches, and it prevents bacteriophodopsin in purple membranes from penetration between the nanoislands where the local enhancement of the electric field of exciting light wave is maximal.

Keywords: SERS, silver nanoisland films, bacteriorhodopsin, rhodamine 6G

Acknowledgements. This work was financially supported by the Ministry of Education and Science of the Russian Federation (Projects 11.G34.31.0020 and 16.1233.2014/K) and the Government of the Russian Federation, Grant 074-U01. For providing the samples of purple membranes and assistance with SERS experiments the authors are grateful to Vitaly Polovinkin, Valentin Borshchevskiy and Valentin Gordeliy (Laboratory for Advanced Studies of Membrane Proteins, Moscow Institute of Physics and Technology, Dolgoprudny, Russia; Institut de Biologie Structurale J.-P. Ebel, Grenoble, France; Institute of Complex Systems (ICS), ICS-6: Structural Biochemistry, Research Centre Juelich, Juelich, Germany; Institute of Crystallography, University of Aachen (Rheinisch-Westfälische Technische Hochschule), Aachen, Germany.

УДК 543.424.2, 538.97

ГКР СПЕКТРОСКОПИЯ МОЛЕКУЛ БАКТЕРИОРОДОПСИНА, АДСОРБИРОВАННЫХ НА СЕРЕБРЯНЫЕ НАНООСТРОВКОВЫЕ ПЛЕНКИ

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Аннотация. Представлены результаты экспериментов по поверхностно-усиленной рамановской (ГКР) спектроскопии молекул бактериородопсина в пурпурных мембранах, адсорбированных на самоорганизованных серебряных нанооостровковых пленках. Островки серебра формировались на поверхности натрий-кальциевого силикатного стекла в результате ионного обмена в расплаве смеси нитратов серебра и натрия, $Ag_{0,05}Na_{0,95}NO_3$, при температуре 325°C в течение 20 минут и последующем отжиге в водородной атмосфере при температуре 250°C в течение 10 минут. Типичные пленки состояли из расположенных близко друг к другу островков размером 20–30 нм. В ходе тестирования в качестве подложек для ГКР спектроскопии островковые пленки позволили наблюдать соответствующие характерные линии комбинационного рассеяния от высушенной капли водного раствора родамина 6G с концентрацией 10^{-6} моль/л. Аналогичным образом изготовленные подложки были использованы для исследования ГКР спектров бактериородопсина в пурпурных мембранах, диспергированных в воде. Нами были зарегистрированы линии комбинационного рассеяния в областях 1000–1020 $см^{-1}$, 1150–1220 $см^{-1}$ и 1530–1570$см^{-1}$. Подложки со нанооостровковыми пленками серебра позволили зарегистрировать характерные линии комбинационного рассеяния бактериородопсина с концентрацией лишь на порядок меньшей, чем в случае стеклянной подложки. Таким образом, усиление сигнала комбинационного рассеяния оказалось существенно меньшим, чем в случае родамина 6G. Предположительно, это связано с тем, что молекулы бактериородопсина упакованы в белковый кристалл, что не позволяет им проникать в промежутки между островками серебра, где локальное усиление электрического поля падающей волны максимально.

Ключевые слова: ГКР, серебряные нанооостровковые пленки, бактериородопсин, родамин 6G

Благодарности. Работа выполнена при финансовой поддержке Министерства образования и науки Российской Федерации (гранты №№ 11.G34.31.0020 и 16.1233.2014/K), а также государственной финансовой поддержке ведущих

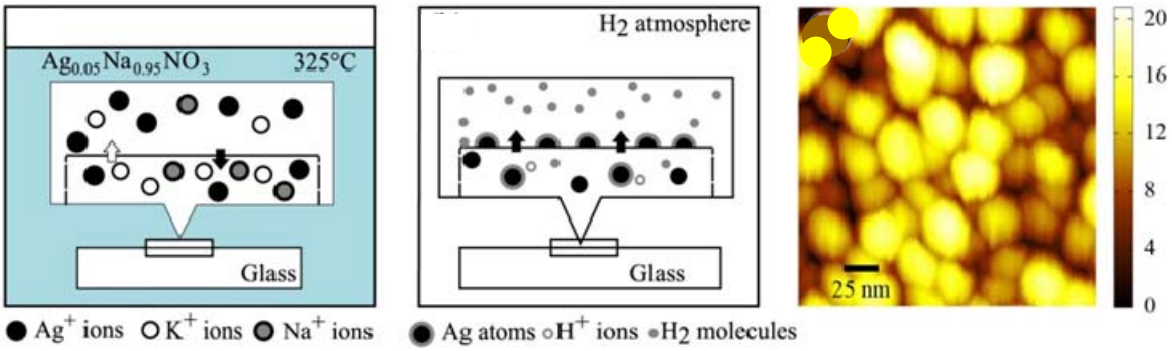
университетов Российской Федерации (субсидия 074-U01). За предоставление образцов пурпурных мембран авторы благодарны Виталию Половинкину, Валентину Борщевскому и Валентину Горделию (Лаборатория «Перспективные исследования мембранных белков» Московского физико-технического института, г. Долгопрудный, Россия; Институт структурной биологии, Гренобль, Франция; Институт сложных систем ICS-6: структурная биология, Исследовательский центр Юлиха, Юлих, Германия; Институт кристаллографии, Университет Аахена (Rheinisch-Westfälische Technische Hochschule), Аахен, Германия).

Introduction

Bacteriorhodopsin (BR) is a light-driven proton pump from *Halobacterium salinarum*: it captures light energy and uses it to move protons through the membranes out of biological cells [1]. This gives the possibility for the conversion of light into chemical energy. In nature BR exists in so-called purple membranes (PM) which have patches consisting of BR incorporated into lipids with an approximate mass ratio BR: lipids equal to 3:1 [1]. BR is well studied by various biophysical methods [1–4] and belongs to the class of membrane proteins, which are of great interest to the pharmaceutical industry [5, 6]. This paper is devoted to the observation of surface-enhanced Raman scattering (SERS) [7] from BR in PM. The SERS is assisted by self-assembled silver nanoisland films formed on the surface of an ion-exchanged glass.

Silver nanoisland films: fabrication and characterization

The technique used to fabricate the silver nanoisland film on a glass substrate includes two steps (Fig. 1): silver ion exchange into glass followed by thermal treatment of the glass in a hydrogen atmosphere [8]. We used commercially available Menzel microscope slides [9] as glass substrates. These soda-lime glass substrates are placed for 20 minutes in $Ag_{0.05}Na_{0.95}NO_3$ ion exchange batch at the temperature of 325°C (Fig. 1, a). This temperature is sufficient to activate the diffusion of alkali ions out of the glass and their replacement with silver ions from the melted silver-sodium nitrate. This results in the enrichment of the subsurface layer of the glass with silver ions.



a b c

Figure 1: Silver nanoisland film fabrication: ion exchange between glass and molten salt (a); thermal treatment in hydrogen atmosphere (b); Typical AFM picture of the fabricated nanoisland film used for SERS measurements (c)

After the ion exchange the glass substrate is treated for 10 minutes in hydrogen atmosphere at the temperature of 250°C (Fig. 1, b). Hydrogen diffuses into the subsurface glass layer, reducing silver ions via the following reaction:

$$\equiv \text{Si-O-Ag}^{+} + \frac{1}{2}\text{H}_2 \rightarrow \equiv \text{Si-O-H}^{+} + \text{Ag}^{0}.$$

Due to the low solubility of neutral silver in the glass the silver atoms coagulate and form nanoparticles. In this soft mode of hydrogen treatment the nanoparticles are being formed on the glass surface rather than in the bulk of the glass and the self-arrangement of the formed nanoparticles results in the formation of the silver nanoisland film. A typical atomic force microscopy (AFM) image of the surface of the glass film with nanoislands is shown in Fig. 1, c. The size of the nanoislands obtained in the regime described above is about 20–30 nm.

Silver nanoisland films as SERS substrates. For the measurements of the Raman spectra we used the confocal Raman spectrometer Horiba LabRAM™ HR UV-VIS-NIR and a frequency-doubled continuous-wave Nd:YAG laser with a wavelength of 532 nm as a light source. The laser beam was focused at the sample surface with a Mitutoyo M Plan Apo 50x/0.55 objective lens. The beam waist was approximately 1.5 μm.

To perform the Raman experiments, we deposited drops of the analytes dissolved in water in different concentrations on the surface of the prepared glasses and dried them at room temperature; the diameter of the so formed spots of the analytes was about 7 mm. All the experiments were carried out with analytes deposited both on the nanoisland film and virgin glass surface.

To verify the ability of the prepared silver nanoisland films to enhance the Raman signal, we used rhodamine 6G (R6G) dissolved in water in the concentration of 10^{-6} M. The spectrum shown in Fig. 2 was

acquired from R6G on silver nanoisland film at 30 s exposition and averaged over 20 measurements while using 20 nW exciting laser radiation power at the sample. The observed characteristic peaks at 612 cm^{-1}, 772 cm^{-1}, 1362 cm^{-1}, 1510 cm^{-1} and 1647 cm^{-1} are in good agreement with literature data [10]. Similar BR SERS results were reported earlier in our paper related to silver nanoislands formed on the surface of a silver-containing phosphate glass using out-diffusion technique [11]. The reference spectra obtained from R6G deposited on a virgin SERS-inactive glass substrate demonstrated a strong luminescent background which gave no possibility to distinguish any Raman peaks and to estimate the degree of the enhancement provided by the nanoisland film.

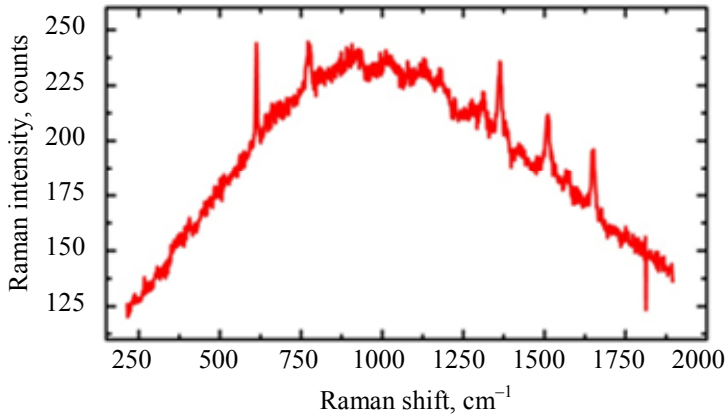


Figure 2: SERS spectrum from R6G on nanoisland film acquired at laser power of 20 nW, 30 s exposition.

Raman scattering of bacteriorhodopsin. The main goal of this study was to register SERS spectra of BR in PM assisted by fabricated silver nanoisland film. The experiments have been carried out using BR in concentrations of 0.48 and 0.048 mg/ml, see Fig. 3. We used 200 μW exciting laser power and acquired the spectrum within 50 s averaging it over 2 measurements. For the sample with a concentration of 0.48 mg/ml (Fig. 3, a) the observed Raman peaks at 1000–1020 cm^{-1}, 1150–1220 cm^{-1} and 1530–1570 cm^{-1} are in good correspondence to the literature [12]. Raman peaks were registered using both virgin glass and the nanoisland film. Fig. 3, a, clearly shows that the nanoisland film makes it possible to resolve more Raman peaks than the virgin glass.

Raman measurements were performed using BR in concentration of 0.048 mg/ml. We have demonstrated that the spectrum obtained using the non-SERS-active glass substrate contains only background without any distinguishable peaks (Fig. 3, b). At the same time the Raman spectrum of BR acquired using the nanoisland film shows vivid peaks around 1020 cm^{-1} and 1530 cm^{-1}.

It is important to mention that BR molecules are packed in PM patches consisting of approximately 1000 molecules [1]. The thickness of each patch is about 5 nm and corresponds to the size of BR molecules, whereas the lateral size of each patch is of the order of several hundreds of nanometers. So BR molecules in PM are not located between the nanoislands where the local enhancement of the electric field in the light wave is maximal. These results in a weaker Raman signal were compared to the ones obtained for R6G molecules.

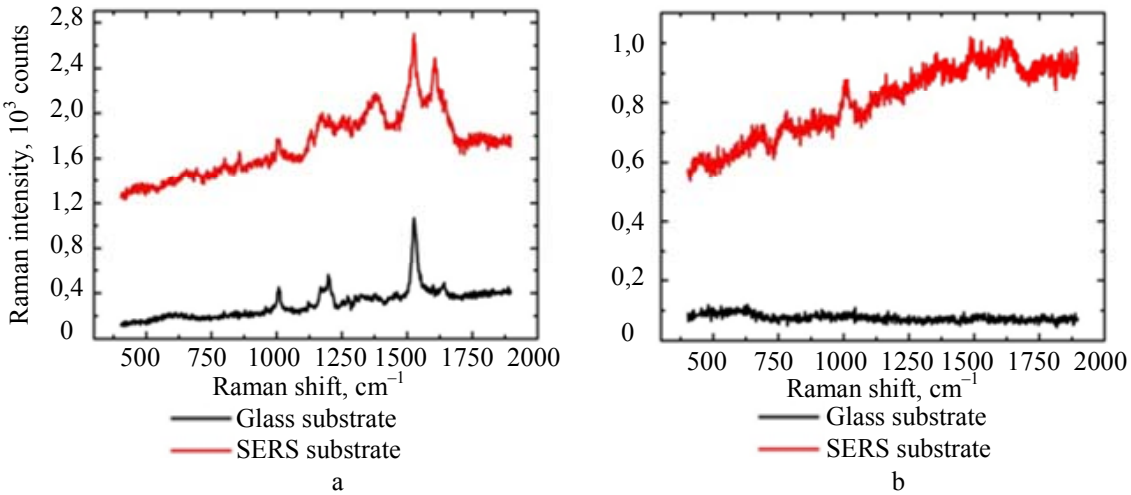


Figure 3. Raman spectra of BR in the concentrations of (a) 0.48 and (b) 0.048 mg/ml. The black and red curves correspond to reference Raman spectra of BR on virgin glass substrate and SERS spectra of BR deposited on the nanoisland film respectively. The spectra were acquired at laser power of 200 μW, with an exposition of 50 s

Conclusion

Finally, we have fabricated silver nanoisland films by means of hydrogen/metal ions reactive diffusion in glasses followed by the transport of neutral metal to the glass surface. This gave the possibility to obtain uniform nanoislands with the size of the order of tens of nanometers.

We have performed SERS measurements of R6G in order to verify the capability of the fabricated silver nanoisland films to deliver enhancement for Raman spectroscopy. The BR in PM spot formed after drying a 5 μl drop with BR in PM in concentration of 0.48 mg/ml demonstrated selective enhancement of Raman peaks already observed in experiments without SERS substrate. For an order of magnitude with lower concentration of BR in PM the usage of the fabricated silver nanoisland film gives the possibility to resolve characteristic Raman peaks in contrast to the virgin glass substrate.

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Принято к печати 26.06.14
Accepted 26.06.14