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Biophysics**DYNAMIC PLASMON ENHANCED RAMAN SCATTERING IN BIOMOLECULES
AND WHOLE BLOOD ON Ag NANOPARTICLES****O.K. Gasymov, O.Z. Alekperov, R.B. Aslanov**

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Surface enhanced Raman spectroscopy (SERS) is a highly sensitive method to characterize biomolecules down to the single molecule level. Previously, various methods have been employed to fabricate nanoparticles of Ag and Al to generate plasmon systems for enhanced Raman scattering. Here, we employ a simple method to fabricate Ag nanoparticle surfaces for enhanced Raman scattering. Silk fibroin was used as a paradigm for biomolecules. Dynamic plasmon enhancement of Raman spectra of silk fibroin films was observed. Different runs of the same samples after exposure to the laser light for a certain period of time show dramatic changes in intensities of the vibrational bands. Relative intensities as well as number of the resolved bands are significantly differ from each other within spectra. Possible mechanism of such selective enhancement of various bands and their dynamic nature are discussed. Ag nanoparticle system was also tested on complex body fluid system, whole blood. Preliminary data indicate that localized plasmons created by Ag nanoparticles enhance Raman bands by various degrees. Feasibility to use the plasmon enhanced Raman spectra of the whole blood for diagnostics is discussed.

Keywords: surface enhanced Raman spectroscopy, nano-plasmon, silk fibroin, Ag nanoparticles

Introduction

Silk fibroin and its composites with different biopolymers are widely applied in various areas of biotechnology [1-3]. Particularly, fibroin films obtained from aqueous solution of fibroin have extensively been used to fabricate biomaterial with drastically different physicochemical properties. Fibroin fibers show about 60% crystallinity that composed of beta-pleated sheets. Dissolution of fibroin in aqueous solution results in a loss of beta-sheet structure. Fibroin films obtained from these solutions retain non-beta-sheet structure. Treatment of the fibroin films with methanol induces beta-sheet structure, fraction of which can be in a range of 0.43-0.56 [4]. Beta-sheet content of fibroin films can be controlled with water vapor annealing at various temperature. This method is a good alternative to methanol treatment.

Fibroin films are excellent biocompatible materials that are extensively used in biotechnology. Bone like material can be obtained by mineralization of fibroin films. In contrast, transparent fibroin films have a great potential

to be used in artificial cornea replacement [5]. Many physicochemical properties of the films depend on secondary structure of fibroin molecules. Therefore, investigation of the structure of fibroin films obtained by various procedures is of great interest. Particular interest is to characterize structure of thin fibroin films that are not accessible to study with regular spectroscopic techniques.

Raman spectroscopy is a powerful technique to characterize secondary structure of proteins in both solution and solid states. Surface enhanced Raman spectroscopy is very sensitive to characterize proteins down to a single molecule level. Localized plasmon obtained with metallic (mostly Ag or Al) nanoparticles is responsible for enhancement of Raman intensities. Various methods are available to fabricate nanoparticle surface for Raman spectroscopy. Here, we employ a simple method to fabricate Ag nanoparticle surfaces for enhanced Raman scattering. Dynamic plasmon enhancement of Raman spectra of silk fibroin films on Ag nanoparticle surface was observed. Exposure to

the laser light for a certain period of time stimulates dramatic changes in intensities of the vibrational bands. Different runs of the same samples reveal drastically dissimilar spectra. Relative intensities as well as number of the resolved bands significantly differ from each other within spectra. Possible mechanisms of such selective enhancement are discussed.

Materials and methods

Materials

A standard purification procedure was used to obtain fibroin from silk fibers [1]. Silk fibers were boiled for 30 min in 0.02 M Na_2CO_3 to wash of soluble protein sericin. Then, fibroin fibers were washed thoroughly with distilled water and then dried. Dried fibroin fibers were dissolved in 9.5 M LiBr solution and then dialyzed (molecular weight cut-off 12 kDa) against water for 3 days at 4°C. Fibroin solution was filtrated and then kept at 4°C. The solution was used as needed during 2 weeks. Fibroin solution was applied to various surfaces to obtain films.

Freshly taken peripheral whole blood samples were directly applied to the glass or Ag nanoparticle surfaces for Raman spectroscopy.

IR spectroscopy

IR spectra of silk and fibroin fibers were obtained with FTIR spectrometer (VERTEX 70V, Bruker) using ATR accessory. The spectral resolution was 4 cm^{-1} .

Raman spectroscopy

Raman spectra of silk and fibroin fibers as well as fibroin films at various surfaces were obtained with confocal Raman spectrometer (Confocal Raman spectrometer, Nanofinder30, Tokyo Instr., Japan) at a wavelength of 532 nm. The magnification factor of the objective lens was 40.

Atomic force microscopy (AFM)

The surface roughness of the Al mirror was assessed using AFM (SmartSPM 1000, AIST-NT, Japan). The software provided by the company was used for analysis of AFM images.

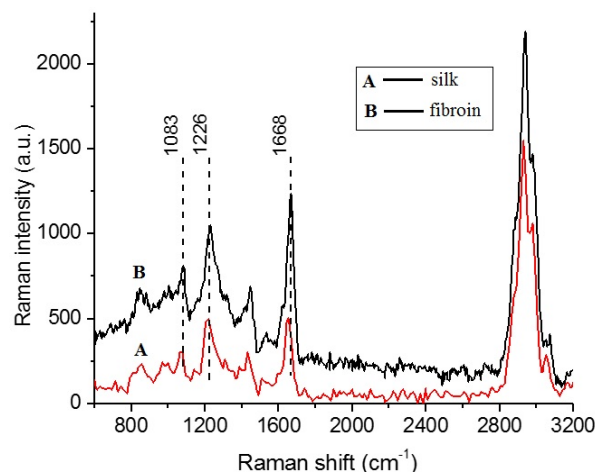


Fig. 1. Raman spectra of silk (A) and fibroin (B) fibers

Fabrication of Ag nanoparticle surface

Ag nanoparticles were created by application of dilute solution of conductive silver paste (Germany) on glass surfaces. Wide angle X-ray diffraction patterns of the surfaces indicate size distribution of about 37 nm for Ag nanoparticles.

Experimental results and discussion

The primary materials used in this study for film formation were previously characterized by FTIR and Raman spectroscopies [6]. Amide I bands observed about 1623 cm^{-1} were used to calculate secondary structure of silk proteins. A vibrational band at 1623 cm^{-1} is indicative of beta structure [7]. Silk and fibroin fibers contain 40% and 45%, respectively, beta structures.

The Raman spectra (fig. 1) of silk and fibroin fibers show peaks at 1083 cm^{-1} , 1226 cm^{-1} and 1668 cm^{-1} , which are characteristic for beta-structure [8]. Fibroin fibers show higher Raman intensities compared to that of silk.

Previously, surface-enhanced infrared spectroscopic ellipsometry (SEIRSE) on fibroin films have been observed on Al surface [6]. The disordered Al nanoislands (shown by AFM data) enhanced the signal approximately 10 times in the spectral interval 1500-1800 cm^{-1} . Non-uniform enhancement of the vibrational bands was observed across the spectra. Features that are characteristic for both electromagnetic and chemical enhancement have been observed in SEIRSE [6].

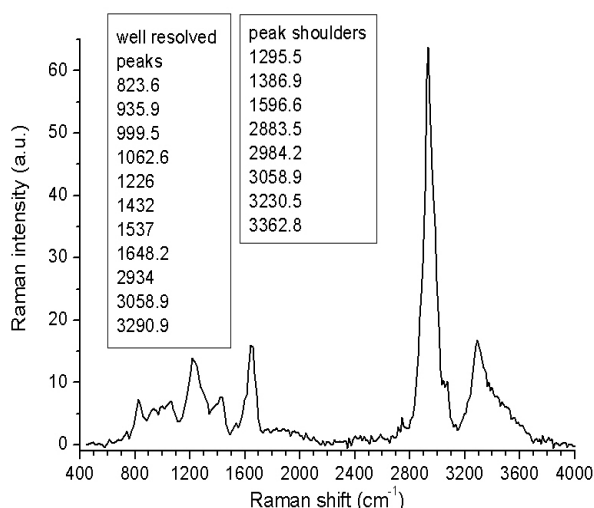


Fig. 2. Baseline corrected Raman spectrum of silk fibroin on Al mirror. (laser power: 10 mW, integration time= 20s. Spectrum was normalized to integration time of 0.14 s for comparison with other spectra

Raman spectrum of silk fibroin on Al mirror shows some enhancement with well resolved bands (fig. 2). The characteristic bands related to secondary structure of proteins are obvious. To increase the Raman intensity significantly, fibroin films were applied to Ag nanoparticle surface. Wide angle X-ray scattering (data not shown) revealed nanoparticles with diameter about 37 nm. Nanoparticles of this size are well suited for the laser light enhanced Raman spectroscopy. As can be seen, fibroin film on Ag nanoparticles shows tremendous enhancement. In some cases, Raman spectra of thin film of fibroin on Ag nanoparticle surface have displayed 10^6 fold augmentation compared to that of the films on glass surfaces. Distance dependence of Raman intensity indicates plasmonic nature of the enhancement (fig. 3). After exposure of samples to the laser light (532 nm, 10 mW) for 90 s, spectra showed very strong dynamic enhancement compared to that of obtained on Al mirror. Different runs of the spectra at the same condition show selective enhancement of different vibrational bands. In few cases, spectra with strongly mitigated vibrational bands were observed (fig. 3). Two mechanisms of enhancement, electromagnetic (EM) and chemical, are considered for the enhancement on metallic nanoparticle surface [9].

Besides that, selective enhancement will be observed for the groups whose dipole moment derivative components perpendicular to the surface. General shape of the vibrational bands and concurrence of some fine features (fig. 3, dotted lines) indicate that well resolved vibrational bands are not artificial but originated from existent groups. Dynamic nature of the enhancement can be result of thermal motion induced by laser light. It has been shown that maximal plasmonic enhancement will be observed in the situation where nanoparticles of 30 nm diameter will be spaced by 1 nm from each other. Thermal motion of nanoparticles may periodically create those situations. On the other hand, some protein groups (for example, side chains) may preferentially oriented by thermal motion of protein molecules. Therefore, two instances may contribute to the dynamic enhancement. First, thermal motion periodically sets favourable distances (about 1nm) between Ag nanoparticles. Second, thermal motion of the protein periodically aligns various protein groups in favourable orientation.

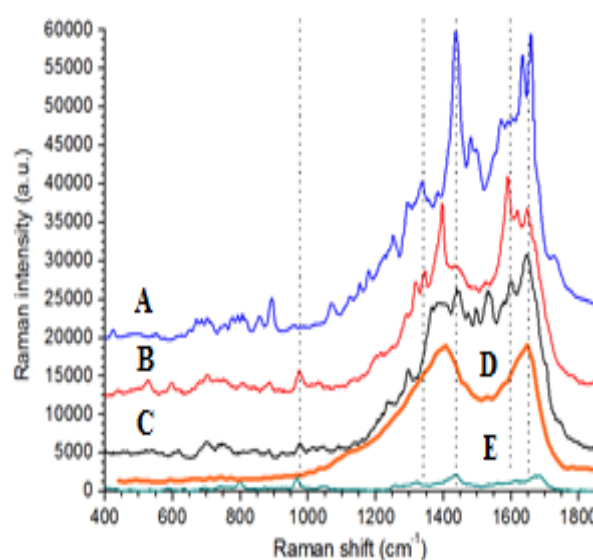


Fig. 3. Dynamic Raman spectra. Baseline corrected Plasmon enhanced Raman spectra of silk fibroin on Ag nanoparticle surface (A,B,C,D represent different runs). E represents the spectrum taken 0.6 mkm above the enhancement point. Spectra are vertically shifted for better visual guidance. Laser power 10 mW, spectra taken after 90 s of laser exposure. Integration time= 0.14 s

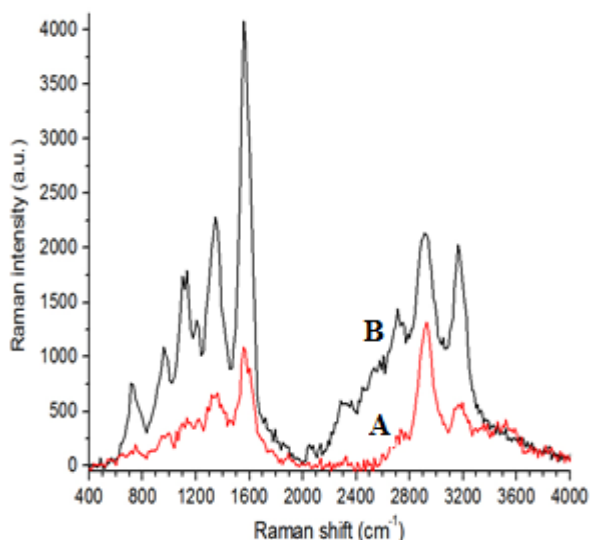


Fig. 4. Baseline corrected Raman spectra of whole blood dried on glass (A) and Ag nanoparticles (B). Laser power 2 mW, integration time= 10 s

The chemical mechanism is considered to be a second enhancement mechanism in Raman scattering. In this case, substrate-nanoparticle interaction creates charge-transfer state, which modifies electronic state of the substrate. Enhancement factor for the chemical mechanism is thought to be about 100, significantly less than electromagnetic enhancement [9].

On the other hand, this mechanism works on very short distances, nearest vicinity of the nanoparticles. Therefore, the contribution to the enhancement by the chemical mechanism can be separated by distance dependent Raman scattering.

It is evident from figure 3 that along Amide bands of the protein (for example around 1640 cm^{-1}), vibrational bands of some side chains are also well resolved. The peaks at 852 cm^{-1} , 1214 cm^{-1} and 1619 cm^{-1} could be assigned for Tyr residues. The peak 1011 cm^{-1} belongs to the Phe residues. Detailed analysis of the spectra will be published elsewhere. Here we would like to show that dynamic enhancement significantly increase a number of the re-

solved bands in Raman scattering by enhancing different peaks at each run.

New plasmonic system was tested on much more complex system, whole blood (fig. 4). It is evident that Raman spectrum of whole blood is significantly enhanced on Ag nanoparticle surface compared to that of produced on glass. Preferential enhancement as well as better resolved vibrational bands are obvious.

Almost all bands of Raman spectra coincide with each other in two systems. This system could be used for diagnostics. Indeed, the Raman peak for glucose (1131 cm^{-1}) [10] is well resolved in Raman spectrum of whole blood on the Ag nanoparticle surface.

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Ag NANO-ZƏRRƏCİKLƏRİNDƏ BİOMOLEKULLARDA VƏ BÜTOV QANDA DİNAMİK PLAZMON GÜCLƏNMİŞ RAMAN SƏPİLMƏSİ**O.K. Qasimov, O. Z. Ələkbərov, R.B. Aslanov**

Səth gücləndirilmiş Raman spektroskopiyası biomolekulları, hətta vahid molekul səviyyəsində tədqiq etmək üçün çox həssas metoddur. Əvvəllər plazmon yaratmaq üçün Ag və Al nano-zərrəciklərinin hazırlanmasının müxtəlif metodları tətbiq olunub. Bu sistemlər plazmon gücləndirilmiş Raman səpilməsi üçün istifadə olunub. Bu işdə biz güclənmiş Raman səpilməsində istifadə etmək üçün Ag nano-zərrəciklər səthinin fabrikasiyasının çox sadə metodunu tətbiq etmişik. İpək fibroini biomolekul nümunəsi kimi istifadə olunmuşdur. İpək fibroini təbəqələrində dinamik plazmon gücləndirilmiş Raman hadisəsi müşahidə olunub. Lazer işığının müəyyən vaxt ərzində təsir etdikdən sonra, eyni nümunənin müxtəlif ölçmələri rəqsi zolaqlarda intensivliyin güclü dəyişməsinə səbəb olur. Nisbi intensivliklər və ayırd edilmiş zolaqların sayı spektrlərdə bir-birindən kəskin fərqlənir. Bu nəticələrə səbəb ola bilən müxtəlif mexanizmlər təklif olunmuşdur. Göstərilmişdir ki, Ag nano-plazmonları qanın Raman zolaqlarını effektiv olaraq müxtəlif dərəcədə gücləndirir. Sonuncu qan diaqnostikasında istifadə oluna bilər.

Açar sözlər: səth gücləndirilmiş Raman spektroskopiyası, nano-plazmon, ipək fibroini, Ag nano-zərrəcikləri

ДИНАМИЧЕСКИЙ ПЛАЗМОН, УСИЛЕННЫЙ РАМАНОВСКИМ РАССЕЯНИЕМ В ЦЕЛЬНОЙ КРОВИ И БИОМОЛЕКУЛАХ НА Ag НАНОЧАСТИЦАХ**О.К. Касумов, О. З. Алекперов, Р. Б. Асланов**

Поверхностно усиленная Раман-спектроскопия является чувствительным методом для исследования биомолекул, даже на уровне одной молекулы. Ранее, для изготовления наночастиц плазмонов Ag и Al были применены различные методы, которые использованы при изучении Рамановских спектров. Для использования усиленного Раман-рассеяния впервые применен простой метод изготовления, который позволяет получить более однородное распределение Ag наночастиц. В пленках шелкового фиброина в предложенной системе наблюдалось усиление Раман рассеяния до 10^6 раз. При больших интенсивностях возбуждения (10mW) обнаружен новый эффект Раман рассеяний, заключающийся в возникновении в зависимости интенсивности различных полос от времени, будто бы случайным образом. Обсуждаются возможные механизмы, приводящие к обнаруженному эффекту. Показано, что наноплазмоны Ag в различных степенях эффективно усиливают Рамановские полосы крови. Последние могут быть применены в диагностике крови.

Ключевые слова: Поверхностно-усиленная Рамановская спектроскопия, nano-плазмоны, фиброина шелк, наночастицы Ag