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EFFECT OF NANOSTRUCTURED METAL SURFACE ON SEIRA SPECTRA OF ALBUMIN AND NUCLEIC ACIDS

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Advantages and disadvantages of the usage of different nanostructured gold surfaces, namely, rough gold and colloidal gold in SEIRA (surface enhanced infrared absorption) experiments are discussed. We have studied SEIRA spectra of biological polymers — DNA and bovine serum albumin. A small enhancement of the IR signal has been registered for multilayers of biopolymers (up to 7 in DNA on rough gold and up to 3 for albumin on colloidal gold) in comparison with small molecules (e. g. up 13 for Gly on rough gold). Alongside of this, we have registered a monolayer of albumin on rough gold. In this case the enhancement was more than 100. The most counspicuons disadvantage registered by us was connected with the influence of the colloidal gold on the DNA conformation. Colloidal gold could change the conformation state of A-DNA inducing the B-A conformation with elements of Z-form. We have found that rough gold does not practically influence the conformation state of DNA in comparison with those on CaF_2 substrate.

Key words: DNA, BSA (bovine serum albumin), SEIRA (Surface Enhanced Infrared Absorption), glycine.

PACS number(s): 78.20.−e, 33.20.Ea.

I. INTRODUCTION

During the last decade spectroscopy of enhanced infrared (IR) signals from molecules located or attached to metal surface or metal particles has been effectively used for biological, electrochemical, surface, sensor and other applications [1–7]. The enhancement of the optical process by a factor of $10^2 \dots 10^{11}$ near rough metal surface (Au, Ag, Fe, etc.) has been already known for twenty years for both optical transitions in adsorbed molecules and the processes, which do not depend on the presence of molecules on metal surface [4]. These processes are surface-enhanced Raman scattering (SERS), surface enhanced infrared absorption (SEIRA), metal-enhanced fluorescence, second harmonics generation, etc. The effect consists in the essential increase of the intensity of transition (e. g. the effective cross-section increases by a factor of $10 \dots 10^4$ for IR absorption) or efficiency of the process near metal surface. For the first time an enhancement of the infrared signal from molecules chemisorbed on Au and Ag surface by a factor up to 10^3 has been registered by Harstein and colleagues in 1980 [2]. In 1991 the effect was named SEIRA by Osawa and Ikeda [6] by analogy with SERS. The explanation of the effect is not simple and includes several mechanisms, namely: (i) increase of the electromagnetic field near a rough metal surface or island metal films, or the so-called electrodynamic mechanism; (ii) increase of dipole transition moment of adsorbed molecules, or chemical mechanism [1,3–5]. A great contribution into the theoretical explanation of surface enhanced effects was made by Kosobukin [4,8].

However, along with doubtless SEIRA advantages (high sensitivity of the method, detection of monolayer film or lesser amount of substance up to 10 pg [1,9], a possibility of determining orientation of the molecular groups, enhancement of intensity of some bands by 10–10^3 times, much improved observation of the bands due to surface selection rules) the method has some disadvantages. One of them is metal surface influence on SEIRA spectra in comparison with conventional FTIR spectra. Indeed, a high enhancement is achieved when absorbed molecules are situated more closely to the metal surface [1,6]. Usually these distances are about tens of angstroms or less. Naturally, small molecules could come closely to the metal surface in comparison with big molecules. In both cases nanostructured gold surface influences the molecule orientation and conformation. Here we present a study of gold surface influence upon vibration modes of DNA and albumin deposited on rough gold and colloidal gold surfaces.

II. EXPERIMENTAL

A. Materials and methods

In SEIRA experiments gold layers were obtained by vacuum deposition of 99.999 pure Au from molybdenum heater at the rate of 10–15 Å/s at room temperature substrate upon glass supports (TF-1 glass, 20×20 mm) via an intermediate adhesive Cr layer [10]. Before Au deposition, glass surface was cleaned by NH_4OH:H_2O_2:H_2O and HCl:H_2O_2:H_2O solution subsequently, both 1:2:2 by volume concentration during 5 minutes at boiling temperature. Gold surface was rinsed in bidistilled water and dried in a flow of pure nitrogen after gold preparation and before biological molecule deposition. The thickness

of gold layer was within 150–250 Å in different experiments. The Cr interlayer does not exceed 10–50 Å. Size of roughness for gold film was about 50 Å [7,11]. Sodium salt of calf-thymus DNA were obtained from Serva, bovine serum albumin (BSA) was obtained from Fluka and Sigma.

The samples for FTIR and SEIRA experiments were prepared by deposition of substances from 1 mg/ml DNA aqueous solution, 1...5 mg/ml albumin in PBS solution on gold or CaF_2 substrates. For preparation of BSA monolayer we applied the conventional method used for preparing samples in the SPR technique [10], namely we adsorbed BSA from BSA solution on gold/SiO_2 plate, rinsed it with distilled water until we reached the monolayer. Due to the fact that BSA contains free sulfhydryl groups which could form intermolecular bonds with gold, strong interaction between gold and BSA keep the monolayer of BSA on the surface of gold. We had no possibility to create a monolayer of DNA on gold surface without chemical modification of gold surface or/and without attaching to DNA additional molecules or molecular groups. In this case we will enhance this additional molecular layer more strongly than DNA and spectra will be overlapped. So, this method is proper for other than SEIRA studies. In SEIRA it is better to use such a metal surface that has a possibility to form a strong interaction between metal surface and molecules under study as in the case of BSA on gold.

Colloidal gold nanoparticles of the size of 15–400 Å were produced by reduction of Au from $HAuCl_4$ with sodium citrate and the concentration of 12 mg/ml. Then they were mixed with sodium salt of calf-thymus DNA (highly polymerised) aqueous $5{\cdot}10^{-3}$ M solution, kept in refrigerator for 24 hours, precipitated on gold substrate as well as on CaF_2 and dried lyophilically. We prepared the samples mixing 10 μl of colloidal gold per 10 μl of DNA solution. As a reference the same DNA aqueous solution without colloidal gold was used. BSA aqueous solution of 1 ...5 mg/ml concentration was mixed with colloidal gold, stirred and then precipitated on CaF_2.

B. FTIR and SEIRA spectroscopy

IR spectra were collected in the 500–5300 cm^{-1} region with IFS-66 Bruker instrument in conventional transmittance mode for DNA and BSA on CaF_2 substrate and in reflectance-absorbance (RA) mode on gold substrate. The reflectance attachment used in the experiment has the light incidence angle close to 16.5° [7]. Spectra evaluation was performed with Opus 2.2 software. The position of the bands was estimated by the method of second derivative and/or standard method. Accuracy of frequency values in the spectra was equalled to 0.5 cm^{-1} and of absorption values to 0.0005 a.u. We used the normalised spectra to OH stretching vibration in the 3300–3400 cm^{-1} range for comparison of shape of the bands for DNA and normalized to Amid 1 for albumin.

The experimental factors of enhancement (g^2) were determined as a ratio of peak intensity of the bands for substances on gold surface in reflectance mode to those on CaF_2 substrate in conventional geometry.

Principal component analysis was applied to SEIRA spectra of DNA. One principal component was chosen as the ratio of intensity of maximum of the phosphate asymmetrical band (in the 1240–1230 cm^{-1} region) to the intensity of maximum in the 3400–2300 cm^{-1} region, which is assigned to OH stretching vibration. According to M. Shie [12] this component multiplied by 5.25 characterizes the number of water molecules per nucleotide. The second principal component was the ratio of the intensities at 1712 cm^{-1} and 1700 cm^{-1} that is a characteristic of the conformation state of DNA relating to A, B or Z-form. In principal component coordinate system every SEIRA spectra could be represented by one point. The location of these points depends on the value of the principal components. This data give a possibility to determine the conformational type of nucleic acids as it was applied by us for conformation analysis of DNA from viruses [13] and cancer cells [14] earlier.

III. RESULTS AND DISCUSSION

The energy scheme of the mechanism of the enhancement effect is presented in Fig. 1, 2. IR photon excites both adsorbed molecules and plasmons in metal (Fig. 1) at the wing of plasmon resonance band (insertion on Fig. 2).

Then the energy from plasmon vibrations is transferred to the adsorbed molecules to increase molecular absorbance.

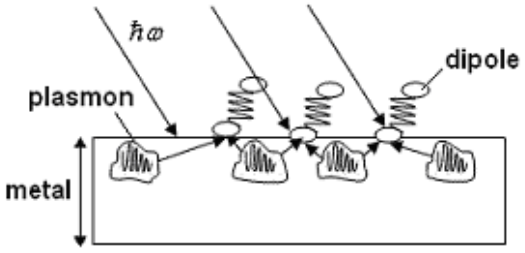

Fig. 1. Mechanism of enhancement on metal surface (SEIRA).

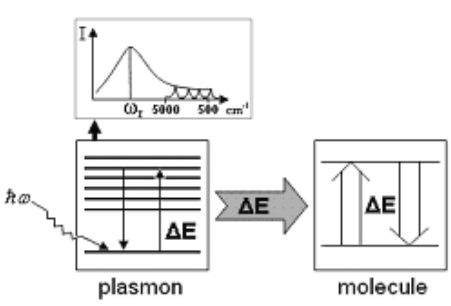

Fig. 2. Energy scheme of enhancement mechanism.

A. SEIRA spectroscopy of DNA on gold

The DNA double strand could adopt different conformation states which has different symmetry, pinch of strand, number of nucleotides per helix, etc. and depends on the environment, humidity, pH, ionic force, etc. [15]. These are well-known A, B (right) and Z (left) form of DNA (Fig. 3). In vibration spectra these forms have distinctive features: the "so-called" marker bands and that is why FTIR spectra could be used for characterizing these conformations [16–17]. However, gold could influence the conformation state of DNA, as well as change their marker band positions. We have modeled the A and B form of DNA on rough gold by changing humidity from 100% (B-form) to 65% (A-form), however, we did not model the Z-form of DNA on rough gold (see Table 1).

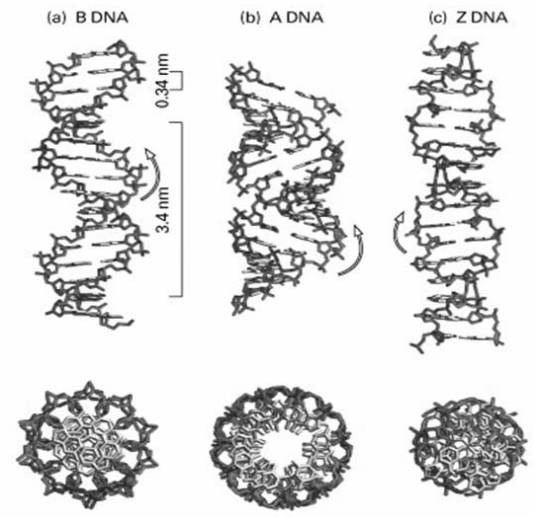

Fig. 3. DNA forms: (a) — B form, (b) — A form, Z form.

Earlier we have repeatedly studied the DNA [14,18,19] on rough gold substrate and concluded that gold substrate did not practically influence the vibration modes and macromolecular conformations of DNA [7,18]. We have found only some minor distinctive features of DNA on gold substrate. The factor of enhancement was registered to 3–7 for different molecular groups; the mass of substance was equal to $1 \ldots 10$ μg. In this case the shift of many vibrations was around 2–3 cm^{-1} for DNA on gold in comparison with DNA on CaF_2, shift of complex stretching of the OH–NH–CH band was 5–10 cm^{-1} (Fig. 4), conformation of the molecules of DNA on CaF_2 was the same as those for DNA on rough gold and close to A-form. The latter could be explained by the fact that the majority of the spectral markers for Na–DNA on the gold substrate are close to the markers of A-form [13] for DNA of 75% humidity.

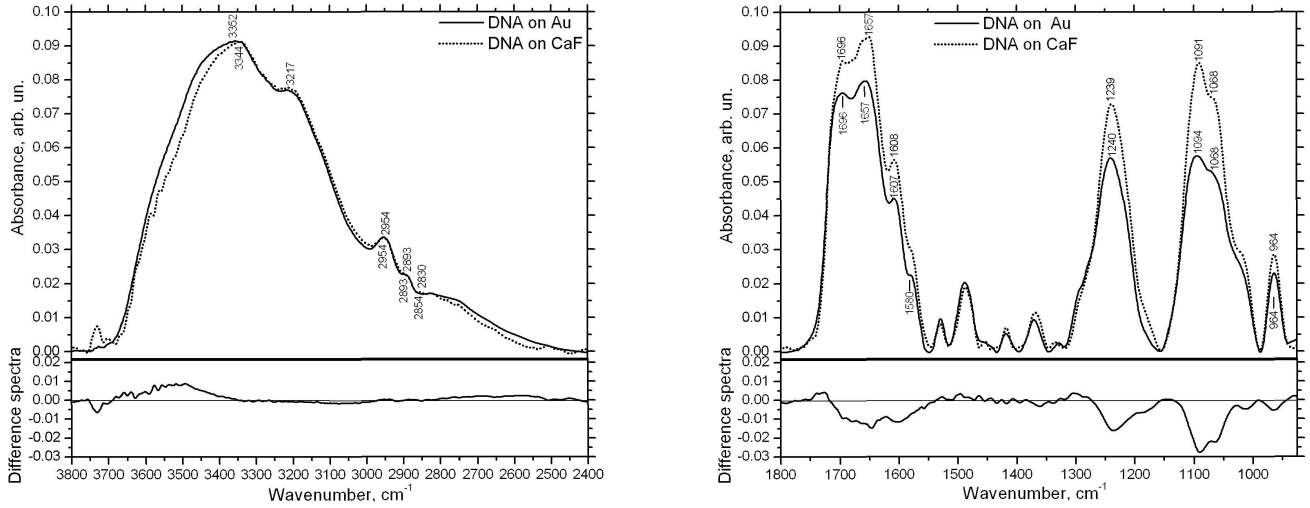

Fig. 4. SEIRA spectra of DNA (normalised to OH stretching vibration) on gold substrate in comparison with DNA on CaF_2 substrate in the 3800–2400 cm^{-1} range (left) and 1800–800 cm^{-1} range (right).

The same is valid for B-form of DNA of 95% humidity [14] and principal component analysis gives a possibility to visualise it [18] (Fig. 5).

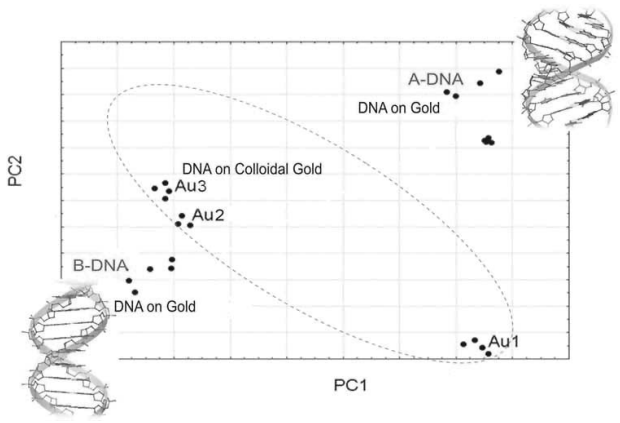

Fig. 5. Principal component analysis of free film of DNA in A and B forms; A and B DNA on gold and DNA with colloidal gold of 10 μl, 20 ml and 30 ml per 10 ml DNA of $5{\cdot}10^{-3}$ M, assigned to Au1, Au2, Au3, accordingly.

Analysing the sugar region of DNA spectra, namely the region lower than 1000 cm^{-1} (Table 1), we must assume that any substrate can strongly influence the sugar conformation of DNA, even in the case when other structural components are not perturbed. In our case, we observed that the gold substrate induces the conformational changes of the sugars' residuals if they come close to gold surface. It is well known that sugars in A-DNA have characteristic features at 805 cm^{-1} and 860 cm^{-1} as well as for B-DNA — at 832 cm^{-1}. The band observed at 834 and 832 cm^{-1} for A and B-form DNA [19], respectively, appears presumably due to reformation of intermolecular H-bonding (NH, OH and CH groups) near the gold substrate [20] as a result of DNA bending at gold peaks. As usual, we observed a band near 830 cm^{-1} for free film of A-DNA prepared on CaF_2 substrate and then taken from substrate. Therefore, this feature is not due to gold substrate but for reformation of bonds near the interface. A similar process was reported earlier by Gaigeot *et al.* [22] in connection with non-coincidence of the data obtained with inelastic scattering of nucleic acid blocks, in solid state and calculations with density functional theory for the spectral region under 900 cm^{-1}. It has been shown that intermolecular H-bonds were formed in the film involving N_1-H and N_3-H groups of the bases giving a strong band at the 830 cm^{-1}.

A-form	B-form	Z-form	A-form on gold	B-form on gold	Assignment
1700–1709	1714–1718	1692–1700	1700	1715	C=O bases
1234–1240	1220–1225	1215	1230–1240	1222–122	PO_2-asym.
1089–1090	1085	1060–1065	1084–1091	1088	PO_2-sym.
860–864	—	864–867	859–860	—	sugar *C3′-endo*
—	841–835	834–838	832–834	834	sugar *C2′-endo*
805–808	—	—	803–805	—	sugar *C3′-endo*

Table 1. Marker models of DNA in A and B forms taken from Schrader [16] and Taillandier [17,21] and DNA on rough gold substrate

B. Effect of colloidal gold on DNA

An essential change in vibration mode position, halfwidth and intensity in SEIRA spectra of DNA-colloidal gold in comparison with FTIR spectra of DNA on CaF_2 have been observed (Fig. 6).

The greatest changes for DNA-colloidal gold system were observed for the OH–NH–CH band, namely, an increase of the halfwidth of OH and NH stretching bands ca. 250 cm^{-1} and their high frequency shift of about 100 cm^{-1}. A decrease in the intensity of the phosphate bands at 1104 cm^{-1} (symmetric stretching) 2.4-fold and at 1240 cm^{-1} (asymmetric stretching) 2-fold; a decrease in the intensity of the base bands at 1800–1550 cm^{-1} (1.3-folds), an increase of halfwidth of phosphate bands about 35–40 cm^{-1} for asymmetric phosphate; a high frequency shift of asymmetric phosphate from 1240 to 1244 cm^{-1}, appearance of shoulder at 1222–1215 cm^{-1}(characteristics of Z form); a high frequency shift of symmetric phosphate from 1093 to 1104 cm^{-1}; a decrease of shoulder band of symmetric phosphate at 1050–1070 cm^{-1}(characteristics of Z form) have been registered as well. The DNA with colloidal gold does

have features of both A, B and Z-form. Indeed, canonical forms of DNA are present here too, especially B and Z-forms (930 cm^{-1}) and there is a reduction of A-form (860 cm^{-1}) [23]. The intensity of the band at 960 cm^{-1} and at the frequencies that are decreased about 1.5 to 2-fold (characteristics of Z form). Principal component analysis was applied for the conversion of the different spectra in separate points. Such a presentation (Fig. 5) shows that the points corresponding to canonical DNA (A and B form) and DNA-colloidal gold have different region of localization in the principal component plane. In this plane the DNA-colloidal gold is close to A-B-form with same elements of Z-form, however, our experimental condition induces A-form.

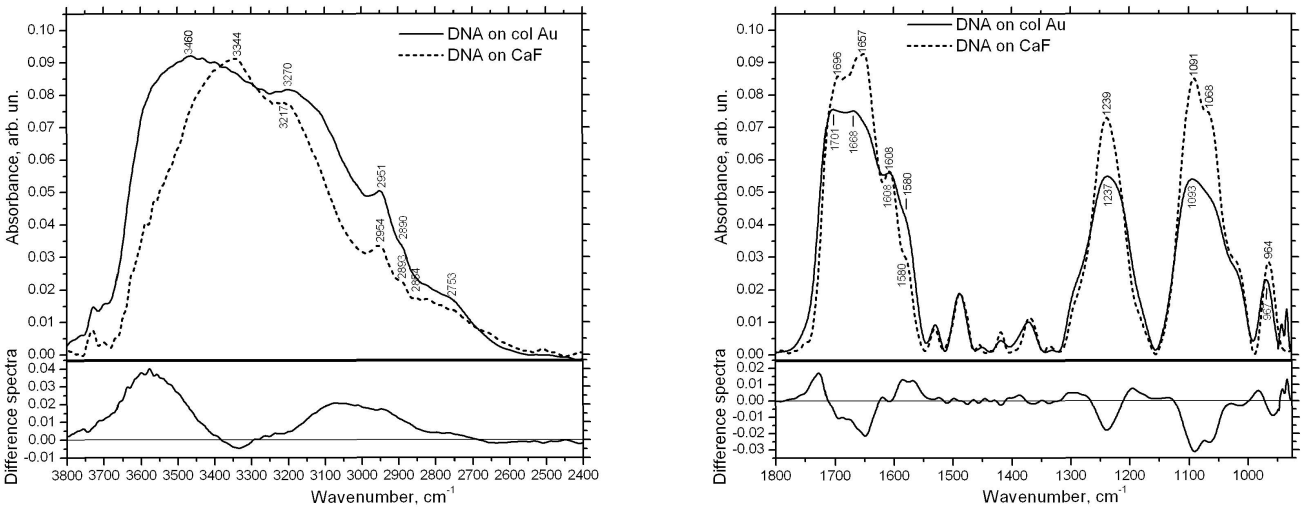

Fig. 6. SEIRA spectra of DNA (normalised to OH stretching vibration) on colloidal gold in comparison with DNA on CaF_2substrate in the 3800–2400 cm^{-1} (left) and 1800–800 cm^{-1} (right).

C. BSA on gold and colloidal gold

1. Multilayer BSA coverage on gold.

Characteristic features of any proteins in vibrational spectra are Amid (A, B, I, II, III, IV, V, VI, VII) bands with the vibration NHCO unit (Fig.7). The amid I vibration, absorbing near 1650 cm^{-1}, arises mainly from the C=O stretching vibration with minor cotributions from the out-of-phase CN stretching vibration, CCN deformation and the NH in-plane bend. The latter is responsible for the sensitivity of the amide I band to N-deuteration of the backbone. The amid I vibration is hardly affected by the nature of the side-chain. It depends, however, on the secondary structure of the backbone and is therefore the amide vibration that is most commonly used for secondary-structure analysis.

The amid II mode is the out-of-phase combination of the NH in-plane bend and the CN stretching vibration with smaller contributions from the CO in-plane bend and the CC and NC stretching vibration with smaller contributions from the CO in-plane bend and the CC and NC stretching vibrations. As for the amide I vibration, the amide II vibration is hardly affected by side-chain vibrations but the correlation between secondary structure and frequency is less straightforward than for the amide I vibration.

The amide III is the in-phase combination of the NH bending and CN stretching vibrations with small contributions from the CO in-plane bending and the CC stretching vibration. In polypeptides, the composition of this mode is more complex, since it depends on side-chain structure and since NH bending contributes to several modes in the 1400 to 1200 cm^{-1}region. Contributions of backbone and side-chain vibrations vary considerably which makes the amide III vibration less suited for secondary structure analysis.

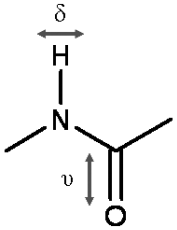

Fig. 7. Structure of NHCO group of any protein.

We have studied the enhancement of BSA on gold and on colloidal gold surface and found that the main marker band of protein-Amid 1 does not show a big enhancement — 2.2 for colloidal gold (Fig. 8) and 1.3 for rough

gold (Table 2). This is in a good agreement with the data obtained by Kuhle et al [24]. They registered an enhancement factor for Amid 1 equaled to 1.4. Unfortunately, this band is important for conformation analysis of proteins. However, some other bands could show more enhancements — up to 5. Another disadvantage of the SEIRA study of BSA on gold is connected with the absorbance dependence on solution concentration [25]. We have not observed absorbance dependence for DNA in the 10^{-2}–10^{-4} M region concentration and for BSA solution of up to 1–5 mg/ml where Bugger–Beer low is valid for Amid A, Amid 1, Amid 2. Our study showed that colloidal gold is a better enhancer than rough gold for BSA multilayer films. In some cases the SEIRA spectra of the BSA-colloidal gold system have registered an evident shift of Amid I band from 1651 to 1588 cm^{-1} caused by the overlap of spectra of protein and colloidal gold [25]. It should be taken into account in studies of BSA with colloidal gold.

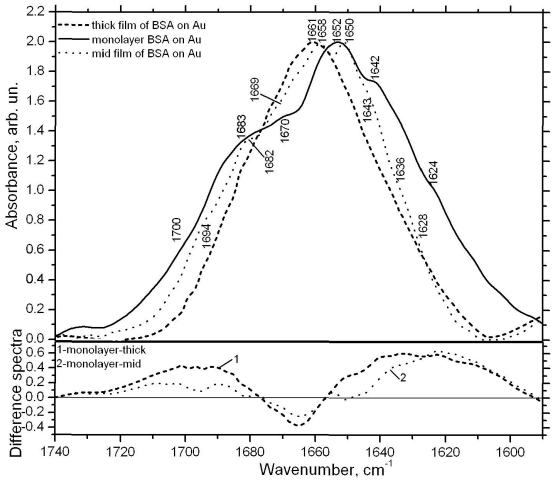

Fig. 8. SEIRA spectra of BSA monolayer, thin and thick layer in the region Amid I band (1750–1600 cm^{-1}).

DNA on Au [18]			Guanine on Au [11]			BSA on Au and (colloidal gold) (Fig. 9)			Gly on Au [26]		
Band position cm^{-1}	g^2	Band assignment	Band position cm^{-1}	g^2	Band assignment	Band position cm^{-1}	g^2	Band assignment	Band position cm^{-1}	g^2	Band assignment
1653	2.9	C=O, C=N, N=H	1633	5.0	def NH, strCN	1660 (1654)	1,3 2,2	Amid I	1585	7.9	COO^- as str
1230	2.4	PO_2^-	1417	6.2	def CNH, str CN	1547 (1545)	1.5 2,1	Amid II	1499	12.6	NH_3^+ sym def
1084	2.1	PO_2^-	1372	6.0	str CN, str CC, def CNH	1302	2.0	Amid III	1333	11.7	CH_2 def, NH bend
963	2.4	C–C, C–O	1313	7.3	def NCH	1245	1.9	Amid III	1409	5.7	COO^- sym str
831	2.0	C_2 endo	1119	3.1	def CNH, str CN	—	—	—	1134	3.2	CH_2 bend, NH_3^+ rock

Table 2. Experimental factor of enhancement for DNA, BSA on gold in comparison with guanine and glycine.

2. Monolayer of BSA on gold.

SEIRA has allowed us to register a monolayer of BSA on rough gold (Fig. 9) that confirms a possibility to reach a big enhancement (more than 100) in the case of monolayer or less. It is impossible to obtain and register a monolayer film on CaF_2 substrate in any FTIR mode.

The spectra of monolayer were compared with the spectra of mid and thick BSA film on gold substrate. Some specific differences in the spectra of monolayer and thicker films were observed.

The first by the halfwidth of Amid 1 monolayer is wider about 15–22 cm^{-1} in comparison with other layers and second, by the shape of the band was changed in comparison with those for a thick film. Namely, the following positions of absorption bands were registred in the spectra of monolayer (in brackets the frequency for mid film is shown): α-helix — 1652 (1650); β-sheet — 1624 (1628), no band (1636, this band position corresponds

also to the deformation vibration of water), 1642 (1643), 1670 (1669); unordered coil — no band (1658), turns 1700 (1694), 1683 (1682) cm^{-1}. This means a strong interaction between protein and gold as well as the rearrangement of contributions of the absorption bands attributed to the various types of protein conformation, i. e. α-helix, β-sheet, unordered coil, turns. This situation has complicated the fitting procedure and conformation analysis of BSA.

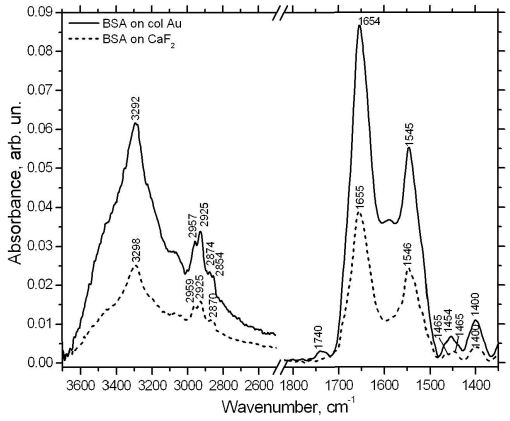

Fig. 9. SEIRA spectra of BSA on colloidal gold in comparison with BSA on CaF_2.

IV. CONCLUSIONS

1. It is really small (less than 10) an enhancement for complex molecules as BSA and DNA in the case of a multilayer coverage and more then 100 for the monolayer of BSA (for DNA we could not reach it due to the fact that DNA covalently bound with gold only with additional molecules). For small molecules, even for multilayer coverage, enhancement reach to 10–20 and as a rule is more than those for complex molecules.

2. Colloidal gold could change the conformation of DNA in contrast with rough gold in the case of DNA multilayer coverage. However, for the enhancement of multilayer coverage of BSA, the colloidal gold is a better enhancer.

3. A high enhancement has a correlation with more numerous spectra distortion.

ACKNOWLEDGMENTS

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ВПЛИВ НАНОСТРУКТУРОВАНИХ ЗОЛОТИХ ПОВЕРХОНЬ НА SEIRA СПЕКТРИ АЛЬБУМІНУ ТА НУКЛЕЇНОВИХ КИСЛОТ

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У статті обговорено переваги та недоліки використання різних наноструктурованих золотих поверхонь, а саме, шорсткого й колоїдного золота в SEIRA (Surface Enhanced Infrared Absorption) експериментах з підсилення інфрачервоного поглинання біологічних полімерів — ДНК та альбуміну металевою поверхнею. Незначне підсилення ІЧ сиґналу зареєстровано для багатошарової плівки біополімерів на золотій поверхні, а саме, для ДНК на шорсткому золоті — близько 7, для альбуміну на колоїдному золоті — 3. Для мономерів, або простих молекул, коефіцієнт підсилення був більшим, зокрема для ґліцину на шорсткому золоті становив близько 13. Значне підсилення ∼100 вдалося зареєструвати для моношару альбуміну на шорсткому золоті. Найбільший недолік використання ефекту SEIRA пов'язаний із впливом колоїдного золота на конформацію молекул ДНК. Колоїдне золото може нав'язувати конформацію, у якій наявні маркерні смуги A–B та Z-форми, на відміну від шорсткого золота, що практично не впливає на конформаційний стан ДНК.