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Surface enhanced infrared absorption of nucleic acids on gold substrate

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Abstract. Data on surface enhanced infrared absorption (SEIRA) of nucleic acids deposited on the metal surface have been obtained in the experiment in FTIR reflectance mode. As metal surface, we used Au of 200-500 Å thickness on glass substrate. Roughness of Au was about 50 Å. In our experimental conditions, the enhancement factor of SEIRA was 3 to 5. We obtained different enhancement factors for different vibrations of nucleic acids. Application of this method to tumour nucleic acid gave a possibility to reveal some structural peculiarities of their sugar-phosphate backbone and those after application of anti-cancer drugs.

Keywords: surface enhanced infrared absorption (SEIRA), RNA, DNA, resistant cancer, sensitive cancer, gold substrate, FTIR reflectance, anti-cancer drugs.

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1. Introduction

The effect of surface enhanced infrared absorption (SEIRA) is well known during last 20 years [1]. It consists in the essential increase (by factor $\sim 10^2$) of the intensities of some IR bands of the molecules adsorbed on metal particles or surfaces. It can be accompanied by increase of intensities of some bands or even decrease of intensities of other bands (which can be characterized as «selection rules»). This effect has been registered first by Hartstein et al. in 1980 [2], similarly to the SERS effect, however only recently it was named as SEIRA and got practical application in the last decade [3–7], mainly due to intensive study of biological molecules [6]. An enhancement factor, which could be reached with SEIRA, equals to 100...1000. The interpretation of this effect similar to more known SERS (surface enhanced Raman spectra) effect includes at least two mechanisms such as local enhancement of external electric field near the rough metal surface due to excitation of surface plasmons and specific increase of the polarizability of the molecules at their adsorption on metal surface [8].

2. Methods and materials

FTIR methods, when applied for structural studies of biological molecules, have essential sensitivity; however, in modern genetic and biochemical applications, where only very small amounts of materials are available, there is a necessity to have increased sensitivity. For this reason, any enhancement of the signal is very important. Nucleic acids belong to the materials whose isolation requires expensive materials and time, so enhancing the FTIR absorption even by factor 3-5 gives one a possibility to enhance both sensitivity and accuracy of the analytical methods. An application of metal rough surface as substrate for studying absorbed molecules could lead to enhancement of the FTIR signal in reflectance or attenuated total reflectance modes. The enhanced FTIR signals from vibrations of nucleic acids deposited on noble metal surface have been registered in the experiment with reflectance attachment (Fig.1). As metal surface, we used Au of 200 to 500 Å thickness on glass substrate. Roughness of Au was not greater than 50 Å. The samples of genomic DNA and total fraction of RNA isolated from

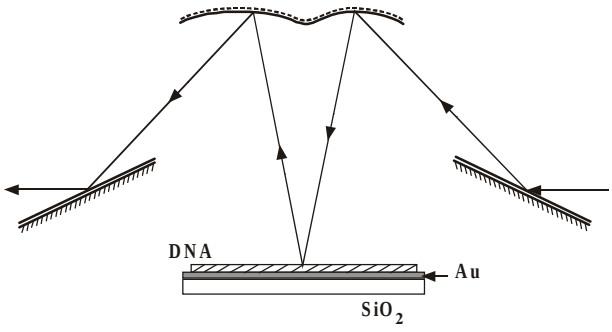

Fig. 1. Geometry of SEIRA experiment.

human brain tumours of different stages of malignancy - astrocytoma (II grade of malignancy) and glioblastoma (IY grade of malignancy) as well as of total fraction of DNA from Carcinoma Guerina cells of Wistar line rats. Changes in the structure of DNA caused by application of cisplatin and doxorubicin as anticancer drugs to these two types of cancers were a subject of investigation, too. The DNA was isolated from cancer cells of rats exposed by 9.5 mG/kG doxorubicin or 6 mG/kG cisplatin during 1 hour. The samples of DNA from aqueous solution were deposited on the Au surface of 200-500 Å thickness and then were liophilically dried. All samples were kept in closed boxes with P_2O_5 water adsorbent. Nucleic acid concentration was about 1μg/μl. As reference DNA and RNA we used the total fraction of nucleic acids isolated from the same type of normal tissue of Wistar rats.

The reflectance attachment used in the experiment has the light incidence angle close to 16.5°. The FTIR spectra were registered in the 380–5300 cm^{-1} region with IFS 48 Bruker instrument. Deconvolution and decomposition of the bands has been performed with Opus-2.2. program. The positions of the bands have been estimated using the method of second derivative. The enhancement factor of SEIRA for different vibrations was calculated as ratio of integral areas of corresponding bands in SEIRA and conventional geometry (Table 1).

The microphotograph of the Au surface used as substrate for SEIRA was obtained by atomic force microscope (AFM). We used tapping mode of AFM imaging, using a commercial Nanoscope IIIa (Digital Instrument, Santa Barbara, CA). Scans were performed in the tapping force mode using commercially available AFM tips (made of silicon nitride). Scanning frequency was approximately 1 Hz in all experiments.

Table 1. The SEIRA enhancement factor of different vibrations for nucleic acid.

Reference spectrum	Au, 200 A°		Au, 300 A°		Au, 400 A°		Band assignment
Band position, cm^{-1}	Band position, cm^{-1}	Intensity enhancement factor	Band position, cm^{-1}	Intensity enhancement factor	Band position, cm^{-1}	Intensity enhancement factor	
3350	3359	4.3	3354	2.0	3362	1.3	O-H , N-H, C-H
1655	1653	2.9	1649	1.4	1654	1.3	C=O,C=N, N-H of Adenine, Thymine, Guanine, Cytosine
1528	1530	3.0	1529	1.1	1529	1.2	C=N Adenine, Cytosine
1490	1486	2.6	1489	1.0	1488	1.1	Def. C-H
1419	1418	2.8	1418	1.0	1418	1.1	Def. C-H
1373	1370	3.8	1371	1.6	1371	1.5	Def. N-H, C-H
1238	1230	2.4	1230	1.0	1230	1,1	Str. PO_2^- asym
1089	1084	2.1	1084	0.8	1084	0.9	Str. PO_2^- sym
964	963	2.4	964	0.9	963	1.1	C-C, C-O deoxyribose
892	889	1.7	892	0.9	889	0.6	C-C, C-O deoxyribose
833	831	2.0	829	0.8	831	1.3	$C_{2'}$ – endo conformation of shugar

2.1. SEIRA experiment

The adduced below theoretical interpretation of the effect is connected with enhancement of external electric field due to excitation of local (surface) plasmon vibrations at essential curvature of rough surface or on metal particles. In such systems, according to [8], the electric field in the point of space r could be written as follows (all formulae presented here are taken from [8]) :

$$E(r,t) = \exp(-i\omega t)\,[E_0(\omega) + E_1(r,\omega)]. \quad (1)$$

Here ω is frequency, t is time. The additional term $E_1(r, \omega)$ connected with excitation of local (surface) plasmon vibrations. The amplification of the external field can be presented as

$$E_\alpha(r,\omega) = g_{\alpha\beta}(r,\omega)E_{0\beta}(\omega), \quad (2)$$

where $g_{\alpha\beta}(r, \omega)$ is a coefficient of amplification.

For the matrix elements of dipole moment of the transition between the states i (with energy e_i) and f for the adsorbed molecule of nucleic acid, we have

$$\langle f|d\alpha(r)|i\rangle = h_{\alpha\beta}^{(fi)}(r)\,\langle f|d_{0,\beta}|i\rangle \ , \quad (3)$$

where $\langle f|d_0|i\rangle$ is the matrix element of dipole moment of transition for isolated molecule.

According to classical approximation, the matrix elements of the Hamiltonian of the interaction of the adsorbed molecule with field are:

$$\langle f|H(r,\omega)\,i\rangle = -\langle f|d_\alpha(r)|i\rangle E_\alpha(r,\omega) = \\ = -h_{\alpha\beta}^{(fi)}(r)g_{\alpha\gamma}(r,\omega)\langle f|d_{0,\beta}|i\rangle E_{0,\gamma}(\omega) \quad (4)$$

Having performed substitution from Eq. (4) into the expression

$$\omega_{fi}(r,\omega) = (2p/\hbar)\,|\langle f|H(r,\omega)|i\rangle|^2\,\delta(h\omega - \varepsilon_f + \varepsilon_i)$$

for the rate of the $i \to f$ transition, multiplied the result by the energy $h\omega$ of absorbed quantum, and divided by the density of the incident energy flow $cE_0^2/8\pi$ (where c – the light velocity), we obtain the expression for the effective cross-section of light absorbance by adsorbed molecule:

$$\sigma_\alpha(r,\omega) = \frac{16\pi^2\omega_{fi}}{cE_0^2}\,|\langle f|H(r,\omega)\times \\ \times|i\rangle|^2\delta[\,h(\omega-\omega_{fi})] \sim \sigma_\alpha^{(0)}|g(r,\omega)|^2|h(r)|^2\,, \quad (5)$$

where $\sigma_\alpha^{(0)}$ is cross-section for the case when metal is absent ($\mathbf{d} = \mathbf{d}_0$, $\mathbf{E} = \mathbf{E}_0$). The right hand part of the expression (5) is the estimation of the cross-section as to its order of magnitude; this all formulae presented here are taken from [8] estimation shows the dependence of the cross-section on the (averaged over tensor indices) coefficients of amplification of the electric field g and dipole moment h of the transition near the metal surface. The influence of the last factor is probably not essential in some experiments where the moments of transition for the absorbed molecules remain unchanged. In the most cases amplification of the electric field g^2 could be expressed by the proportional to $\varepsilon'/\varepsilon''$ of the metal surface [8].

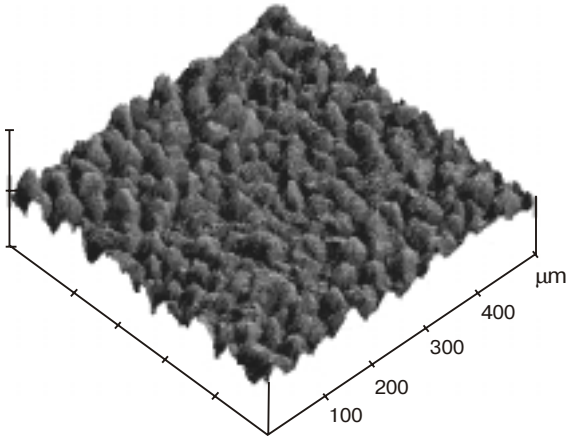

Fig. 2 AFM image of the gold surface used in SEIRA experiment. The size of roughness of the surface is 50 Å.

The estimation shows that the enhancement of the effect is increased in the case when the characteristic size of roughness of the surface is close to 50-100 Å. In our experiment, we used gold substrate with similar size of roughness (Fig. 2) and obtained enhancement of SEIRA by factor 3-5 for different vibrations (Fig. 3). We assume that this is not maximal enhancement, which could be obtained in the system.

3. Results and discussion

In the spectra of DNA and RNA, we have two wide bands near 1090 (Phosphate I) and 1230 cm^{-1} (Phosphate II)

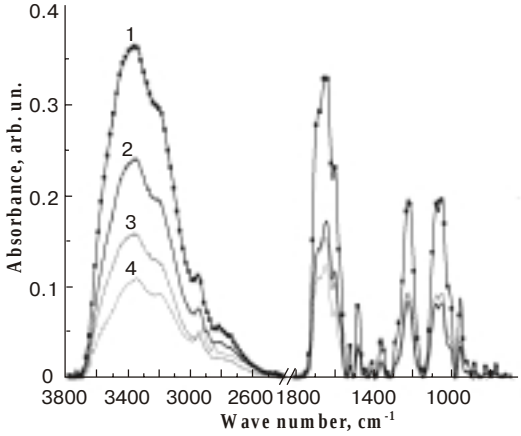

Fig. 3. SEIRA spectra of DNA: 1 – on gold substrate with 200 Å thickness; 2 – 300 Å, 3 – 400 Å, 4 – on CaF_2 substrate.

attributed to vibrations of sugar-phosphate backbone. Each of these bands consists of several overlapped bands. Decomposition enables one to obtain the positions and intensities of these bands. Earlier [9] we studied RNA isolated from rats (normal and with tumours) and found a number of features specific for tumour RNA. In [9] we have registered the FTIR absorbance spectra of tumour brain nucleic acids on CaF_2 and Si substrates, so we had no possibility for studies of Phosphate I and sugar vibrations in the 700-900 cm^{-1} region due to restricted transmittance of these substrates. The application of SEIRA to nucleic acids on Au/SiO_2 substrate gave the possibility to obtain good quality spectra in 1100-600 cm^{-1} region with enhancement factor 3-5 and to reveal additional features of molecular structure of tumour nucleic acids (Fig. 4). In the FTIR spectra of tumour RNA on Au/SiO_2, we found appearance of the band at 1147-1155 cm^{-1} (Fig. 5, curves 2,3,4) with increasing intensity in the case of more malignant tumour. In the absorption spectra of reference RNA, this band is practically absent. The halfwidth of Phosphate I band was drastically greater in RNA from tumour cells (Fig. 5, curve 2,3,4) in comparison with those for reference RNA. The redistribution of intensities of main peaks of Phosphate 2 band (near 1243 and 1223 for reference RNA) and high frequency shift of the peaks in the case of the most malignant tumour (Figs 4,5 curve 2) have been observed in tumour RNA. More vibration states were calculated under the decomposition of these phosphate bands in the case of tumour RNA as well as DNA. The peculiarities of tumour DNA looks like as RNA with less prominent manifestation.

In the region of sugar moieties of tumour RNA, we have registered not only north-type (C_3-endo) sugar conformation, but south-type (C_2-endo), too. Assignment of sugar conformations was made according to [10,11]. For

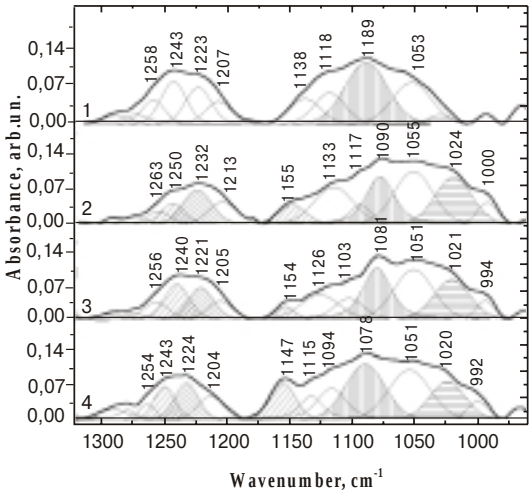

Fig. 5. SEIRA spectra of RNA on the Au substrate: 1 – reference, 2 – glioblastoma (IY grade) 3, 4 – astrocytoma (II grade) in the 960–1320 cm^{-1} region.

glioblastoma (Fig. 4, curve 2), we found both these conformations with approximately equal intensities. In other tumour RNA (Fig. 4, curve 3, 4), we have registered suppression of any sugar vibrations in the 700-900 cm^{-1} region. Arising of the band at the 793 cm^{-1} (Fig. 4, curve 4) could be attribute to guanine in C_3-endo/syn conformation, which appears in Z-conformation of DNA [12].

Under the application of doxorubicin to the sensitive cancer, the structure of DNA in the sugar region looks like that for DNA from normal tissue (Fig. 6). In the case of doxorubicin application, the structure of sugars practically does not change in the resistant cancer cells. A ratio of intensities of Phosphate I and Phosphate II bands

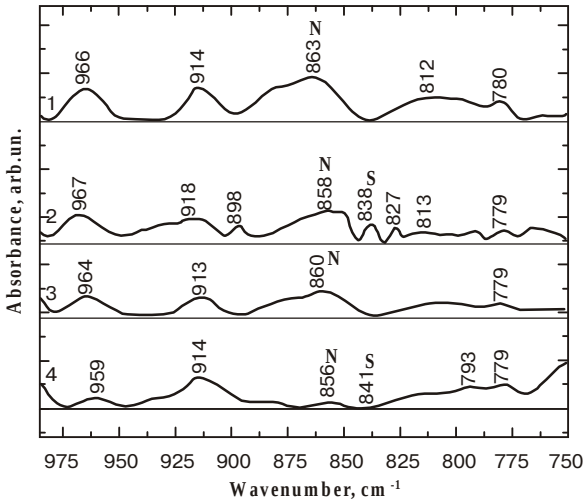

Fig. 4. SEIRA spectra of RNA on the Au substrate: 1 – reference, 2 – glioblastoma (IY grade.) 3, 4 – astrocytoma (II grade) in the750–970 cm^{-1} region.

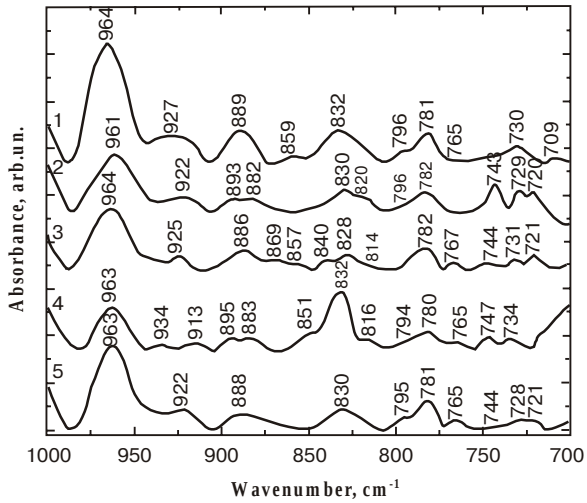

Fig. 6. SEIRA spectra of DNA: 1 – reference spectra, 2 – from resistant tissue, 3 – from resistant tissue after treatment with doxorubicine, 4 – from sensitive tissue, 5 – from sensitive tissue after treatment with doxorubicine.

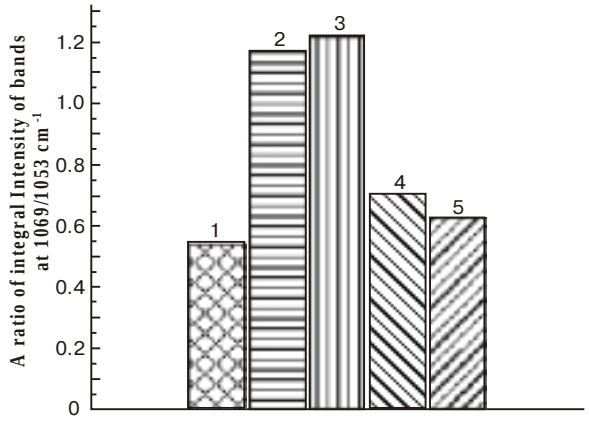

Fig. 7. A ratio of integral intensity of bands at 1069 cm^{-1} to 1053 cm^{-1} for DNA: 1 – reference spectra, 2 – from resistant tissue, 3 – from resistant tissue after treatment with doxorubicine, 4 – from sensitive tissue, 5 – from sensitive tissue after treatment with doxorubicine.

in the resistant cancers does not change after treatment by drugs, but in the case of sensitive tumours, this ratio changes by factor 1.5 or more. In the region of OH-NH-CH stretching vibrations, strong H-bonded NH vibration band near 2600 cm^{-1} becomes stronger for the resistant cancers after treatment. The spectrum of resistant tumour DNA looks like the spectrum of DNA from normal tissue, and their changes after application of anti - tumour drugs are negligibly small. Thus, the DNA of resistant tumour cells could be characterised as rigid structure. The structure of sensitive cancer DNA seems to be flexible, and after application of anti-cancer drugs it drastically changes and becomes close to the structure of DNA from normal tissues. The Fig. 6 confirm abovementioned influence of anti-cancer drugs on the structure of sensitive and resistant cancers in the Phospate 1 band for two different C-O vibrations of deoxyribose at 1053 cm^{-1} (ordering structure of DNA) and 1069 cm^{-1} (disordering structure of DNA). Calculating the ratio of integral intensity of contributions of these two vibrations, we can characterize the level of disorder in the DNA structure. We concluded about increasing of disorder in resistant cancers in comparison with sensitive cancers. For DNA from sensitive cancer after treatment, the disorder became less and for resistant cancers it became more after treatment by anti-cancer drugs.

Conclusions

Application of SEIRA to nucleic acids on gold substrate with 200-500 Å thickness and size of roughness 50 Å has shown amplification of absorbance by a factor of 3 to 5 for various vibrational modes of nucleic acids. SEIRA has revealed the structural features of the tumour nucleic acid and those after application of anti-cancer drugs connected with conformations of sugars and phosphate groups in the sugar-phosphate backbone.

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