STIMULATED LOW-FREQUENCY RAMAN SCATTERING IN BROME MOSAIC VIRUS

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Abstract

We experimentally register stimulated low-frequency Raman scattering (SLFRS) in the suspension of brome mosaic virus (BMV) in phosphate buffer with very high conversion efficiency. We identify two components of the SLFRS spectrum as the breathing and quadrupole modes of BMV and determine damping characteristics and gain factors for these modes. We show that, using the core-shell model for BMV and taking into account the influence of the environment, the acoustic properties of individual components of such a composite nanosystem can be determined. Thus, we define the sound velocity in the RNA core of BMV, in view of spectral characteristics of SLFRS.

Keywords: stimulated low-frequency Raman scattering, viruses, brome mosaic virus, acoustics, eigenfrequency, resonance impact.

1. Introduction

Studying viruses is evidently an important scientific and practical task. Viruses are responsible for diseases of people, animals, plants, and periodic epidemic outbreaks; therefore, they are an object of intense study. The ability to quickly and accurately characterize new strains of viruses is important for predicting and controlling such outbreaks, therefore, various diagnostic methods are being actively developed. Even more important for practical purposes is to develop the methods of fast identifying certain viruses. Optics methods are one of the most effective for this aim. Real-time nondestructive identification processes are based on the Raman scattering effect.

Raman spectroscopy methods, such as ultraviolet resonance Raman (UVRR) spectroscopy and polarized Raman spectroscopy, are being actively developed and improved [1]. A compact and convenient platform for virus capture and identification was recently introduced [2], where a real-time nondestructive identification process was based on the surface-enhanced Raman scattering effect. Raman spectroscopy provides an important information on the molecular composition of the systems under study. For determining the morphology in the study of nanoscale (1–100 nm) and submicrometer (100–1000 nm) systems,

the low-frequency Raman scattering (LFRS) can be used [3]. Such powerful tool as LFRS [4] can be a complement to different Raman spectroscopy methods for the identification of various biological systems. Characteristics of low-frequency acoustic vibrations of viruses (as any nanoparticles or submicroparticles) are defined by their own shape, their elastic properties, and the properties of the surrounding medium, and they are independent on the exciting intensity.

Thus, the LFRS use in viruses can provide information on their elastic properties and explain how they change under conditions very close to real ones. This fact makes this method unique and very interesting from the viewpoint of biology and biomedicine. In the case of strong attenuation, where the acoustic impedances of a nanoscale (or submicrometer) object and the environment are slightly different, the LFRS efficiency can be quite low. In this case, SLFRS can be used for studying the morphological properties and for exact definition of natural vibrations of dielectric nanoparticles in suspensions [5, 6]. Of particular interest, there is the measurement of the natural vibration frequencies of nanoscale viruses dangerous to humans, for example, influenza viruses or SARS-CoV-2, with the aim of their subsequent destruction or suppression under optical (two-photon) or microwave resonance irradiation. For these purposes, it is of interest to study plant viruses that are safe for humans and have a similar shape (sphere) [7].

For the first time, the resonant microwave absorption studied in brome mosaic virus (BMV) and tomato bushy stunt virus has been proposed for their destruction [8]. Later a reduction of the tobacco mosaic virus activity was shown after exposure to the microwave field [9]. But due to strong water absorption in the microwave spectral ranges, this method is very difficult to implement in real conditions. To overcome this difficulty, one can use the excitation sources in the visible range, where water is transparent. Due to its high conversion efficiency, SLFRS can be used as a biharmonic pump source for effective resonant impact on viruses.

In this paper, we present the results of experimental investigation of SLFRS excited in the suspension of spherical virus BMV in phosphate buffer. The SLFRS energetic and spectral characteristics are defined. We show that the core-shell model is the most suitable for describing the elastic properties of the system under investigation.

2. Experimental

2.1. Samples

BMV is a small (27–30 nm, 86 S), positive-stranded, icosahedral RNA plant virus belonging to the genus Bromovirus, family Bromoviridae in the alphavirus-like superfamily. The BMV virion is composed of 180 identical subunits of the capsid protein arranged in a $T = 3$ icosahedral geometry. The virion structure was determined by X-ray crystallography with a resolution of 0.34 nm. The capsid subunits exist in three different arrangements forming 12 pentameric and 20 hexameric capsomeres. At the center of each capsomere, there is a channel with 0.5–0.6 nm diameter. The capsid itself has a thickness of 5–6 nm and weighs roughly $3.6 \cdot 10^6$ Da.

Virus particles contain approximately 22% nucleic acid and 78% protein. The genomic RNA does not fill completely the interior of the particle leaving a central cavity of about 8 nm. Molecular weight of the complete virion is approximately $4.6 \cdot 10^6$ Da. The isoelectric point determined by isoelectric focusing is 6.8. The brome mosaic virus virions were purified [10], with slight modifications. Infected leaves of barley (*Hordeum vulgare* L) were blended in 0.1 M phosphate buffer at pH 5.0. The leave sap was filtrated

Fig. 1. Distribution of BMV hydrodynamic radius in phosphate buffer and TEM image.

through cheese cloth incubated for 2 h. at room temperature and subjected to high-speed centrifugation (100,000 g, 2.5 h.; CP100WX, Hitachi). The pellet was dissolved in 0.1 M phosphate buffer at pH 7.0.

2.2. Experimental Setup and Results

SLFRS measurements were carried out using the setup described in [5]. Ruby laser pulses ($\lambda = 694.3$ nm, $\tau = 20$ ns, E_{max} per pulse equal to 0.3 J, $\Delta\nu = 0.015$ cm $^{-1}$) were used for the SLFRS excitation. The length of the active medium (BMV in phosphate buffer) was 10 mm. SLFRS spectra were registered with Fabry–Pérot interferometers with different ranges of dispersion (from 0.3 to 8.3 cm $^{-1}$) simultaneously in the forward and backward directions. All our measurements were carried out at room temperature.

In Fig. 1, we present the hydrodynamic radius distribution of BMV in phosphate buffer obtained by dynamic light scattering with a Photocor Compact Z analyzer. On the right-hand side of Fig. 1, we show the transmission electron microscopy (TEM) image of brome mosaic viruses obtained with JEOL JEM-1400. The peak with a maximum at 16 nm corresponds to the individual BMV viruses. In addition, there is also a peak for larger size (38 nm) that corresponds to the aggregates of viruses. Figure 2 shows the summarized SLFRS spectra obtained for BMV in phosphate buffer in forward scattering geometry.

Peaks with spectral shifts of 0.45 cm $^{-1}$ (13.5 GHz), 0.55 cm $^{-1}$ (16.5 GHz), 0.74 cm $^{-1}$ (22.2 GHz),

Fig. 2. SLFRS spectra measured for BMV suspension in the forward direction at a laser intensity of 0.2 GW/cm 2 with the Lorentzian fit (dashed curves).

1.08 cm⁻¹ (32.4 GHz), and 1.94 cm⁻¹ (58.5 GHz) were registered separately for each exciting pulse with different probability both for the forward and backward scattered waves. Their maximum conversion efficiency was 30%, 45%, 35%, 35%, and 15%, respectively. In order to classify these frequency shifts, it is necessary to determine the vibrational eigenmodes of the virus particles.

For the case of a free continuous isotropic elastic sphere, this problem was solved by Lamb [11]; in his work, two types of vibrational modes were shown, namely, a spheroidal (SPH) mode and a torsional (TOR) mode, as a result of solving the equation of motion under stress free boundary conditions,

$$\rho \frac{\partial^2 \vec{D}}{\partial t^2} = (\lambda + \mu) \vec{\nabla}(\vec{\nabla} \cdot \vec{D}) + \mu \nabla^2 \vec{D}, \quad (1)$$

where $\vec{D}$ is a lattice displacement vector, μ and λ are Lamb's constants, and ρ is the mass density.

The equations obtained for SPH modes read [12]

$$\frac{\tan(qa)}{qa} = \frac{1}{1 - (v_l^2/4v_t^2) q^2 a^2}, \quad l = 0, \quad (2)$$

$$qa j_{l+1}(qa) j_l(Qa) + (l^3 + l^2 - 2l - Q^2 a^2/2) Qa j_l(qa) j_{l+1}(Qa) + (2 - l^2 - l) qa Qa j_{l+1}(qa) j_{l+1}(Qa) = -(Q^2 a^2/2)(2l^2 - l - 1 - q^2 a^2/2) j_l(qa) j_l(Qa) + (l^3 + 2l^2 - l - 2 - Q^2 a^2), \quad l \neq 0, \quad (3)$$

and for TOR modes are

$$\frac{d}{d(Qa)} \left[\frac{j_l(Qa)}{Qa} \right] = 0, \quad l \geq 0, \quad (4)$$

where v_l and v_t are longitudinal and transverse sound velocities, a is the sphere radius, l is the quantum number of orbital angular momentum, and j_l is spherical Bessel functions of the first kind, $v_l q = v_t Q = \omega$.

The SPH modes is a vibration with dilatation, while the TOR modes are characterized by a constant density, and their eigenvalues are inversely proportional to the particle radius R

$$\omega = \xi V/R, \quad (5)$$

where V is the sound velocity and ξ is a dimensionless parameter depending on the relation between the longitudinal and transverse sound velocities.

The eigenfrequencies for both SPH and TOR modes are described by the quantum number of orbital angular momentum l and harmonic n . Viruses are small particles ($D \ll \lambda$) with a fairly spherical shape and, for such cases, only the breathing ($l = 0$) and quadrupole ($l = 2$) spheroidal modes are Raman-active ones [13]. On the other hand, the virus particle is heterogeneous as it consists of the RNA core and the capsid shell suspended in a liquid buffer; to determine the eigenfrequencies, in this case, is a more complex task.

After Lamb, his free sphere model (FSM) was expanded to the cases with an elastic matrix or liquid surrounding of the sphere and also for special cases of inhomogeneity, including the core-shell model (CSM) [14–17]. These data are in good agreement with LFRS as well as SLFRS experiments for various submicrometer and nanoscale systems including biological systems. In Fig. 3, we show the lowest spheroidal eigenmodes estimated for the FSM model, without taking into account the liquid surrounding and with its consideration, as well as the values experimentally obtained.

Longitudinal and transverse sound velocities used in calculations correspond to the values for lysozyme $V_L = 1817$ m/s and $V_T = 915$ m/s, as it is usually accepted for viruses. In Fig. 3, one can see that the spectral shift 1.94 cm^{-1} (58.5 GHz) most likely corresponds to the breathing ($l = 0$) oscillation mode.

Considering the virus as a core-shell particle, the boundary conditions at various interfaces are changing. For free surfaces, zero surface traction is used and, for interfaces between two different materials, the displacement and the associated surface traction are continuous [18, 19]. To obtain the eigenfrequencies of BMV virus, using the CSM method, it is necessary to know the values of sound velocities in the RNA core but, to regret, these data were not available in the literature. Therefore, changing the value of longitudinal sound velocity and assuming the Poisson ratio to be 0.33 and the RNA density $\rho = 1.21\text{ g/cm}^3$, we obtained the eigenfrequency dependence for breathing ($l = 0$) and quadrupolar ($l = 2$) spheroidal modes of virus in liquid media; see Fig. 4.

Fig. 3. Dispersion relations of lowest SPH modes for BMV calculated for the free sphere model (■) and liquid (⊙) environment, with our experimental values shown by dashed curves.

Fig. 4. Eigenfrequency of SPH $l = 0$ and $l = 2$ modes as functions of the longitudinal sound velocity in the RNA core of BMV.

Under these assumptions, the eigenfrequencies coincide with the experimental ones at the longitudinal sound velocity in RNA ranges from 3700 to 3800 m/s, what is comparable to the values for DNA (3400–3800 m/s) [20]. In this case, a spectral shift of 0.74 cm^{-1} (21.3 GHz) corresponds to the quadrupole ($l = 2$) spheroidal mode. It is worth noting that the sound velocity values in the virus particle's core play an important role, as the rigidity of composite core-shell particle could be strongly dominated by its DNA/RNA core rather than the capsid shell [20].

As it was shown [21, 22], the vibrational modes of viruses embedded in a liquid are expected to be severely damped because of a weak acoustic impedance mismatch and viscosity. When considering the medium influence, eigenfrequencies are complex and their imaginary parts are related to the damping time by the ratio $\tau_D = -1/\text{Im}\omega$. For BMV suspension, the breathing mode is much more damped than the quadrupole mode, their damping times are 16 and 59 ps, respectively. That coincides with the obtained SLFRS spectra, where the breathing-mode component is broader and has smaller conversion efficiency than quadrupole mode one. Therefore, the ability to experimentally observe these modes in such conditions is possible due to the stimulated nature of the SLFRS process.

In Table 1, we present lowest eigenfrequencies estimated for different models. The spectral lines corresponding to 0.45, 0.55, and 1.08 cm^{-1} may refer to the presence of aggregates with an average size of 38 nm in the system under study, which was determined by the DLS method; see Fig. 1. It also may correspond to the SPH modes with odd l or the TOR modes, which could be Raman-active ones, taking into account the anisotropy of the virus as mentioned above.

Parameters used in the calculations are as follows: the virus radius is 28 nm, the DNA core radius is 9 nm, the longitudinal and transverse sound velocities for virus or shell in CSM are 1817 and 915 m/s, respectively, the density of the protein coat and RNA core are 1.21 g/cm^3 , the water density is 1.0 g/cm^3 , and the longitudinal sound velocity for water is 1498 m/s.

Table 1. Eigenfrequencies of BMV for Different Models.

Free Sphere cm^{-1}			Sphere + Liquid Frequency/FWHM, cm^{-1}		Core – Shell cm^{-1}		Core – Shell + Liquid Frequency/FWHM, cm^{-1}	
	SPH	TOR	SPH	TOR	SPH	TOR	SPH	TOR
$l = 0$	1.88		1.43/1.42		2.69		1.94/2.08	
$l = 1$	1.25	1.99	0.42/1.76	1.86/0.22	1.51	2.34	1.17/0.65	2.09/0.37
$l = 2$	0.92	0.87	0.59/0.30	0.62/0.48	1.39	1.02	0.74/0.56	0.72/0.56
$l = 3$	1.37	1.34	0.89/0.47	1.00/0.72	1.87	1.47	1.02/0.73	1.07/0.79

The half-width of the SLFRS spectral line is inversely proportional to the square root of the laser intensity, and the expression for the half-width of the line of the scattered component has the form [23].

$$\Delta\omega \sim \sqrt{\frac{\ln 2}{2glz}} \Gamma, \quad (6)$$

where g is the gain coefficient in $[\text{cm}/\text{MW}]$, z is the interaction length in $[\text{cm}]$, Γ is the half-width of the LFRS spectral line, and I is the laser intensity in $[\text{MW}/\text{cm}^2]$. Using expression (6), the experimental values of the spectral width of the SLFRS line, the laser intensity, and the values given in Table 1 for FWHM of the spontaneous scattering line, one can obtain the values of the gain for modes $l = 0$ and $l = 2$. These values are 0.175 and 0.024 cm/MW , respectively. Note that the gain for the mode $l = 0$ is larger than the gain of the stimulated Brillouin scattering for such highly-nonlinear liquid as CS_2 , for which it is 0.15 cm/MW [24].

3. Discussion

Our investigations clearly indicate that SLFRS can be excited with high conversion efficiency in nanoscale biological systems. SLFRS can be used both for spectral measurements that allow identification of the nanoscale systems under study. Also, SLFRS can be used as a source consisting of two spectral lines (biharmonic pumping) that allows selective and resonant impact on biological objects due to the ponderomotive interaction with the exact coincidence of the object's own acoustic frequency with the frequency difference. As it is known, the ponderomotive force acts on a dielectric particle in an external electromagnetic field; it is determined by the following expression:

$$\vec{F} = (\vec{P}\nabla)\vec{E} = n_1^2 \left(\frac{n^2 - 1}{n^2 + 2} \right) r^2 (\vec{E}\nabla)\vec{E}, \quad (7)$$

with $n = n_1/n_2$, where n_1 is the refraction index of the surrounding medium, n_2 is the refraction index of the nanoparticle, and r is the nanoparticle radius.

If the electromagnetic field consists of two waves with the frequency difference Ω , it leads to the appearance of the ponderomotive force component oscillating with Ω ,

$$F \approx E_1 E_2 e^{i\Omega t}; \quad (8)$$

this ponderomotive force will excite harmonic acoustic vibrations in the nanoparticle. If the frequency difference is equal to the particle's acoustic eigenfrequency, an effective impact on the nanoparticle can be realized.

The application of biharmonic pumping in the visible or near-infrared range for the efficient excitation of the biological object's vibrations due to weak absorption in the liquid environment makes it perspective impact tool for such biosystems.

4. Conclusions

As we showed, the model of BMV as a core-shell object in a specific environment is quite suitable both for determining the type of oscillation modes and for estimation of the acoustic parameters of the BMV individual components separately. Taking into account the effect of the environment leading to attenuation, the damping parameters can be used for calculating the gain coefficients for the corresponding vibrational modes.

Study of such plant viruses as BMV by optical methods, in particular, by SLFRS, is very important. This virus has spherical form as many human viruses (influenza viruses, SARS-CoV-2), which cause infectious diseases. Study of plant-virus elastic properties, definition of virus natural vibrational frequencies, and creating of methods of effective impact on them can help in employment of plant viruses as a model nanoparticles to develop methods of the human virus investigation, identification, and impact on them with the aim of their activity decreasing up to destruction.

Acknowledgments

This study was supported by the Ministry of Science and Higher Education of the Russian Federation for Large Scientific Projects in Priority Areas of Scientific and Technological Development under Grant

No. 075-15-2020-774 and by the Russian Foundation for Basic Research under Grants Nos. 18-04-00028_a, 19-02-00440_a, and 19-02-00750_a.

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