Effects of hydration levels on the bandwidth of microwave resonant absorption induced by confined acoustic vibrations

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We found the hydration levels on the capsid surface of viruses can affect the bandwidth of microwave resonant absorption (MRA) induced by the confined acoustic vibrations (CAV). By decreasing the pH value of solution down to 5.2 or inactivating the capsid proteins, we enhanced the surface hydrophilicity and increased the magnitude of surface potentials. Both of these surface manipulations raised the surface affinity to water molecules and narrowed the bandwidths of CAV-induced MRA. Our results validate the viscoelastic transition of hydration shells. © *2009 American Institute of Physics*. [doi:10.1063/1.3254251]

The vibration modes of molecules can be revealed by infrared absorption spectroscopy if their displacements change the dipole moments of molecules. Depending on the bonding strength, the mass of atoms, and the types of vibrations, the resonant absorption frequencies of molecules range from hundreds of terahertz to several terahertz. For collective vibrations of macromolecules such as proteins or virions, the corresponding resonant frequencies will be around terahertz and could be probed by the terahertz or microwave absorption spectroscopy.[1,2] However, in this frequency range, the periods of vibrations are close to or above the persistence time of hydrogen bonding of water molecules. If the surface to volume ratio of macromolecules is large, surrounding water molecules will overdamp the vibrations and smear the resonant absorption feature.[1] Recently, we demonstrated that confined acoustic vibrations (CAVs) (Ref. 3) of viruses can modify dipole moments and result in microwave resonant absorption (MRA).[2] The resonant absorption frequencies correspond to those of dipolar active [SPH, $l=1$] modes.[4,5] The activation of the resonant coupling relies on the core-shell charge structures,[4] which are inherent on the capsid surfaces.[2] Such characteristic absorption peak is rarely found in terahertz spectroscopy on solvated proteins and the detailed mechanism worth a further investigation.

In general, the bandwidth of CAV-related MRA could either be homogeneously broadened by the acoustic quality factors of [SPH, $l=1$] modes or inhomogeneously broadened by the size distribution. Since the size variation of spherical viruses is typically smaller than $\pm 5\%$, the corresponding quality factor of CAV-related MRA (peak absorption frequency over absorption bandwidth) will be larger than 10. But according to a theoretical simulation on polymethylmethacrylate (PMMA) nanoparticles, viscous water could strongly damp the vibration and decrease the quality factor Q_{MRA} of [SPH, $l=1$] modes down to 3–5.[6] For viruses, whose mechanical properties are close to PMMA, the quality factor of CAV-related MRA should thus be dominated by the viscous damping of water. However, in our previous works, the measured Q_{MRA} of inactivated enterovirus 71 (EV71) was above 10, which is obviously larger than the theoretical prediction. To figure out the actual factor that raises the quality factors of CAV-related MRA in viruses, here we prepared purified *Perina nuda* viruses (PnV) (Ref. 7) in Tris buffer and measured their MRA spectra. The CAV-related MRA frequency of PnV was close to that of EV71 and the corresponding Q_{MRA} match the theoretical prediction well at pH $=7.2$. But when we reduced the pH value to 5.2 and enhanced the surface hydrophilicity of PnV, the bandwidth of CAV-related MRA became narrower and the Q_{MRA} was well above the viscosity limitation. As for EV71, the inactivated EV71 also showed a Q_{MRA} value higher than 10 due to a decrease of negative surface potentials. From these results, we find that the surface affinity to waters can affect the quality factors of CAV-related MRA in viruses. Higher hydration levels of hydration shells result in a viscoelastic transition, which reduces the viscous damping of CAV from free water molecules.

According to Lamb's theory,[3] the confined acoustic modes in a free homogeneous sphere can be classified as spheroidal (SPH) and torsional (TOR) ones. Only SPH modes with angular quantum number l equal to 1 are selection-rule allowed to induce dipolar coupling with electromagnetic waves. For stiff TiO_2 and CdSe/CdTe nanoparticles,[8,4] these modes can be revealed by terahertz absorption spectra. For soft virions with 30–100 nm diameters, the eigen frequencies of [SPH, $l=1$] modes will downshift to the microwave frequency range.[2] As indicated in our previous work, such resonant coupling relies on the surface charges and thus the adsorbed counterions of capsid proteins in buffered solutions. The adsorption processes are dynamic and result from the long range Coulomb force. On the other hand, these charged amino acid residues will also form strong hydrogen bonds with water molecules through charge-

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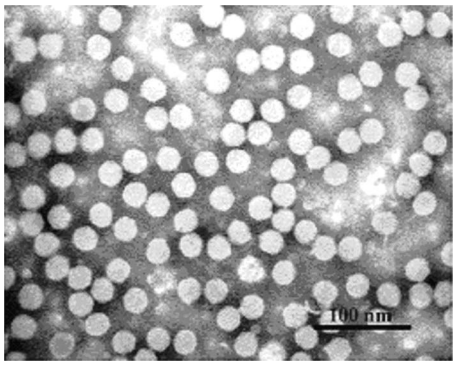

FIG. 1. Negative contrast electron micrograph of isometric particles of PnV. The viral particles are roughly spherical with a diameter of 29.5 ± 0.5 nm.

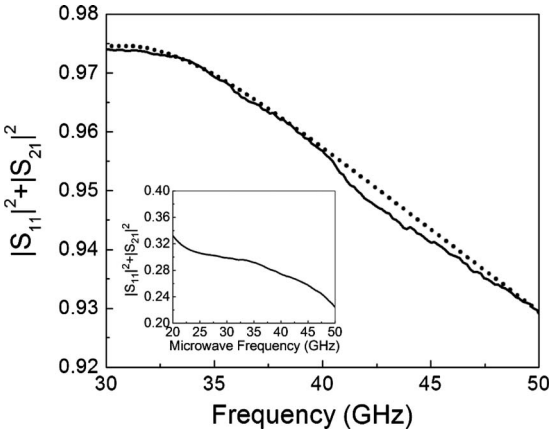

FIG. 2. The attenuation spectra of PnV in pH=7.2 Tris (solid curve) after removing the contribution from buffer (inset). The dotted curve is the fitted dielectric background absorption of PnV.

dipole interactions.[9] Since charge-dipole interactions are stronger than dipole-dipole interactions, the relaxation time of hydrated water molecules around charged cites could be longer than that in bulk water ($\sim 10^{-11}$ s). Depending on the structure and the level of hydration, the persistence time of hydrogen bonds can range from 10^{-11} s to many hours.[10] If the water molecules stay longer than the vibration periods of [SPH, $l=1$] modes, the acoustic impedance of hydration shells will become reactive rather than resistive.[11,12] In consequence, the hydration shells with higher hydration levels could provide better acoustic confinement and result in higher Q_{MRA} than that predicted in Ref. 6.

The PnV is a picornalike insect virus with a capsid composed of 60 copies of four different proteins (CP1, CP2, CP3, and CP4).[7] Only CP1, CP3, and CP4 can reach surfaces and determine the surface hydrophilicity. According to the hydrophobicity indices[13] of amino acid residues, we calculated the residue-averaged hydrophobicity indices of PnV from the protein sequences of CP1, CP3, and CP4.[7] At pH=7.5 the hydrophobicity index of PnV is -4.1×10^{-3}, which is negative but very close to zero. If we made the pH value across the acid dissociation constant pKa of the secondary amines of histidines (pKa=6),[14] the protonation of histidines will drastically decrease their hydrophobicity from −0.65 to −2.28.[13] This change will reduce the hydrophobicity of PnV to −0.03, indicating an enhancement of surface hydrophilicity. To examine the effect of hydrophicility on the CAV-induced MRA, we changed the pH values of buffer from 7.2 to 5.2. The PnV particles were isolated from infected NTU-PN-HH cell lines,[15] purified with CsCl banding, and resuspended in Tris buffer (pH=7.2) with a number density of $3.3 \times 10^{10}/\mu$l. From the transmission electron microscope image, its diameter is 29.5 ± 0.5 nm with a nearly spherical shape (see Fig. 1). The measured zeta potential ζ at the slipping plane of viral colloids is −15.3 mV. Using a dynamic light scattering method, the mean particle size is measured to be ~30 nm, indicating a dispersion of viruses in buffer solutions without severe aggregations. Following the same measurement scheme and data processing procedure conducted in our previous work,[2] we removed the contribution from buffers (inset of Fig. 2) and obtained the microwave attenuation spectra (solid curve in Fig. 2). After considering the background dielectric absorption (dotted curve in Fig. 2) and calculate the insertion loss $1-|S_{11}|^2-|S_{21}|^2$, it shows a weak 0.3% CAV-related MRA at 43.8 GHz (solid gray curve in Fig. 3), which was close to that of the inactivated EV71. The corresponding Q_{MRA} was 5, which agreed well with the theoretical prediction for [SPH, $l=1$] modes of 30 nm PMMA nanoparticles in viscous water.[6] Then we gradually reduced the pH value of the Tris buffer by a titration of HCl. In the pH=6.2 case, under the same viral concentration, the zeta potential of PnV was increased to −8.4 mV due to more adsorptions of anions. The dispersed particle sizes still keep ~30 nm. As we expected, more adsorbed charges Q increased the CAV-related MRA to 4.2% at 46.8 GHz with $Q_{\mathrm{MRA}}=4.5$ (dotted gray curve in Fig. 3).[2] Further decrease the pH value to 5.95, we found no aggregation to occur. More adsorption of anions on capsid surfaces should make the zeta potential closer to the isoelectric point. However, the measured ζ drop to −10 mV, which could result either from more negative surface potentials or less adsorbed anios. This drop of ζ could be due to the fact that the pH value approaches the pKa of the secondary amines of histidines and thus affect the conformations and hydrations of capsid proteins.[14] The corresponding microwave absorption measurement showed a weaker 0.3% peak absorption at 46.8 GHz, confirming a less adsorption of anions. With the decreased anion adsorptions, the CAV-related MRA quality factor Q_{MRA} reduced to 4 (dash-dotted gray curve in Fig. 3). The slightly larger frequency of CAV-related MRA was due to the smaller size of PnV, which might result from the purification steps or the change of pH value. Finally, we made the secondary amines of histidines fully pro-

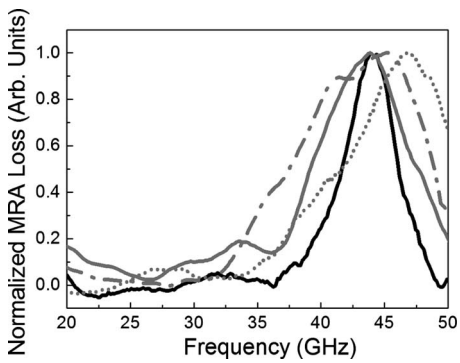

FIG. 3. The CAV-related microwave absorption spectra of PnV in pH=7.2 (solid gray curve), pH=6.2 (dotted gray curve), pH=5.95 (dash-dotted gray curve), and pH=5.2 (solid black curve) buffer. All traces are normalized to their maximal insertion losses after removing the contributions from buffer and background.

tonated by further reducing the pH value of solution down to 5.2. The zeta potential was increased to −8.63 mV with dispersed ~30 nm particle sizes. More adsorption of anions raised the magnitude of CAV-related MRA to 2.5%. The bandwidth of CAV-related MRA was apparently narrowed (solid black curve in Fig. 3) and the Q_{MRA} was abruptly increased to 10.2, which exceeded the theoretically upper limit considering the viscosity of water.[6] From this pH-dependent study, we found that the enhancement of the surface hydrophilicity results in a rise of Q_{MRA}.

Except for the hydrophilicity, another factor that can affect the surface affinity to waters is the surface potentials. To further examine whether this factor also affects the bandwidth of CAV-related MRA, we checked the microwave absorption traces of inactivated EV71 in our previous studies. The inactivated EV71 had a lower −25 mV zeta potential at pH=7.4.[2] Since the number density of EV71 in our previous work is more dilute ($2\times10^9/\mu$l), the normalized magnitude of CAV-related MRA is larger than 5% and comparable to that of PnV. Therefore, more negative zeta potential of EV71 should not originate from less adsorbed anions but from the more negative surface potential. As we mentioned in the introduction, the resulted Q_{MRA} at pH=7.4 were 10.6, suggesting that more negative surface potentials can also attract more water molecules around, enhance the hydration level, and make Q_{MRA} well above the viscosity limitation.[6]

In summary, through the enhancement of surface hydrophilicity or the increase of magnitude of surface potentials, we raised viral surface affinity to waters. The CAV-related MRA bandwidths of viruses were thus narrowed and the corresponding quality factors of CAV-related MRA were well above the viscosity limitation. Our results validate the viscoelastic transition of hydration shells. Higher hydration levels can raise the acoustic confinement in viruses and thus the quality factors of CAV-related MRA. These results indicate that the hydration levels of macromolecules play a critical role in the dipolar coupling of electromagnetic waves with their collective vibrations. This mechanism can be exploited to raise the sensitivity of virus detection with MRA devices. Viruses with similar size and mechanical properties could be distinguished through the bandwidth of CAV-related MRA.

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