Ultrasonic absorption evidence of structural fluctuations in viral capsids

(icosahedral viruses/pH-induced transitions/spontaneous motions in capsids/ultrasonically detected volume change)

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ABSTRACT When the coat protein of the small icosahedral virus, brome mosaic virus, reassembles into capsids, the ultrasonic absorption of the solution greatly increases. Submitting the solution to an ultrasonic field thus appears to reveal spontaneous molecular motions within a protein assembly. Confirmatory evidence of a dynamics of a protein shell comes from measurements on brome mosaic virus at various degrees of swelling and on tomato bushy stunt virus treated with the crosslinking agent glutaraldehyde. The detected fluctuations may be related either with cooperative deformational motions in the capsid or with more localized structural changes. Such structural changes may help liberate the RNA at an early stage of viral infection.

The release by a virion of its nucleic acid is one of the less understood problems in virology, and raises the question of how virus protein shells are destabilized at the start of infection. It therefore seemed of interest to investigate the dissociated protein/capsid assembly equilibrium by using ultrasonic techniques that can provide information on both equilibrium and kinetic characteristics of the molecular processes involved. We have studied two small icosahedral viruses, brome mosaic virus (BMV) and tomato bushy stunt virus (TBSV). In the first, protein–protein and protein–RNA interactions are known to be relatively weak, imparting relatively low stability to the particle. But interactions and stability are stronger in the second virus.

However, the main result of this investigation does not relate to the association–dissociation process. We found that the ultrasonic waves in the MHz range are absorbed much more by capsids than by the dissociated protein. This suggests that spontaneous motions exist within the protein shell, as proposed in preliminary accounts of the results (1–3). We present here evidence to support this interpretation and suggest some biological implications of these findings.

MATERIALS AND METHODS

BMV and TBSV were grown in barley and in Datura stramonium, respectively. Plant proteins present in the sap were precipitated at pH 4.8. BMV was purified by precipitation of polyethylene glycol and resuspended in 50 mM sodium cacodylate buffer, pH 5.0. Swollen BMV was obtained by dialysis against 50 mM sodium cacodylate buffer, pH 7.4, and partially swollen BMV was obtained by dialysis against the same buffer containing 5 mM MgCl₂. BMV protein was obtained by salt degradation of the virus. BMV suspensions were dialyzed overnight in the cold against 50 mM sodium cacodylate-HCl/1 M CaCl₂ at pH 6.7. The RNA precipitated as a calcium salt, which was centrifuged at low speed. The supernatant containing the viral protein was dialyzed against several changes of 50 mM sodium cacodylate-HCl/1 M NaCl at pH 7.5; all the protein obtained in this way was dissociated into dimers of the subunit. For reassociation into capsids, the BMV protein was dialyzed to pH 5.0 against 50 mM sodium acetate/1.0 M NaCl (4). TBSV was purified by three cycles of high and low speed centrifugations. Virus pellets were resuspended in deionized water. Concentrations were measured by ultraviolet absorption spectrometry, using specific extinctions of 5.08 and 5.2 cm⁻¹ (mg/cm³)⁻¹ at 260 nm for BMV and TBSV, respectively, and 0.76 cm⁻¹ (mg/cm³)⁻¹ at 280 nm for BMV protein. All samples used in these studies were homogeneous, as seen by analytical ultracentrifugation in a Spinco model E ultracentrifuge equipped with Schlieren optics.

The absorption of ultrasonic longitudinal waves was measured with an improved Eggers resonator (5) which will be described separately. Two measuring cells were used that cover the frequency ranges 0.6–5 MHz and 3–40 MHz and require 15 ml and 1.2 ml of sample solution, respectively. Unless otherwise specified, all measurements were carried out at 23°C.

Ultrasonic waves perturb the equilibrium between various forms of structures and permit one to characterize molecular relaxation phenomena that occur in the time range 5 × 10⁻¹⁰–5 × 10⁻⁷ s (6). Relaxation occurs when the quantity α/N², in which α is the measured absorption of a longitudinal wave of frequency N, decreases with increasing N values. In simple cases, the relaxation time τ can be derived from the inflection frequency and yields information on the kinetics of the process. When the measurements are performed in aqueous solution, as is the case here, two different structures must have different molar volumes in order to yield relaxation. On the other hand, it is important that, even though the occurrence of absorption requires that some equilibrium between species be disturbed by the ultrasonic wave, the information obtained in the linear range of wave amplitude characterizes spontaneous fluctuations at the molecular level.

RESULTS

BMV Empty Capsids Absorb Much More Ultrasound Than Does Dissociated Protein. When BMV protein polymerized from the dimer into empty capsids, the absorption of ultrasound at frequencies N = 0.6, 0.8, 1.4, and 2.6 MHz increased greatly, as shown in Fig. 1. The midpoint of the dimer–capsid transition, as determined by ultrasonic absorption measurements, occurred in the range 5.7 < pH < 5.8 (varying

Abbreviations: BMV, brome mosaic virus; TBSV, tomato bushy stunt virus.
slightly with the frequency), in accordance with previous measurements of the sedimentation constant (4). Ultrasonic spectra were obtained in the frequency range 0.6-40 MHz for solutions of capsids (4.5 < pH < 5.5), of dimers (6.0 < pH < 9.0), and for the pure solvent. In these two ranges of pH, the protein is fully reassociated in the form of capsids and fully dissociated in the form of dimers, respectively (4). The corresponding spectra are displayed in Fig. 2.

If only protein-solvent interactions can dissipate energy, the absorption would be lower in capsids. Since we observed the contrary, another source of absorption must exist, the contribution of which at least equals the difference between the absorptions of the capsids and of the dimers. We assign this excess absorption to movements within the protein assembly, whereby hydrophobic contacts as well as hydrogen bonds and ionic links between the subunits are periodically perturbed by the sound wave.

The Ultrasonic Absorption of BMV Virions Is No Greater Than the Sum of the Contributions of Their Component Capsids and RNA. In BMV virions, the observed increase of ultrasonic absorption also mainly reflects movements in the protein shell. This follows from the results of Fig. 2 in which the spectra of empty capsids and virions are compared at equal particle concentrations. The measured absorption by the virions nearly equals the sum of the contributions of the protein shell and of the RNA. (The data for RNA are not given here.) We conclude that there can be no large contribution to the absorption resulting from RNA-protein interactions.

Swollen Virions Absorb Less Ultrasound Than Do Compact Virions. In Fig. 3, the amount by which the absorption of the particle exceeds the absorption of the protein dimer is shown to be reduced to less than one-half upon swelling the virion. Virions at an intermediate state of swelling absorb less than the compact virions and more than fully swollen virions. Since protein subunits become less tightly packed when the virus swells (7), the preceding results support our proposed assignment of the excess absorption to protein-protein interactions.

Crosslinking the Proteins in TBSV Diminishes the Absorption. Our assignment is also supported by the reduction of
the excess absorption when virions are treated with a cross-linking agent and the rigidity of the shell is thus increased. In BMV, crosslinking with the bifunctional reagent glutaraldehyde proved not to be feasible at the particle concentration used for these experiments. However, the same reagent did crosslink TBSV. After 24 hr of fixation at room temperature with 2% glutaraldehyde, TBSV still sedimented in the analytical ultracentrifuge as a single component at the same rate as untreated virus. Thus, no crosslinking had occurred between virus particles, but the number of links formed was enough to preserve the structure of the virion under conditions in which it would normally dissociate. Indeed, the glutaraldehyde-treated virus did not dissociate at all in 50 mM Na₂EDTA/100 mM sodium phosphate, pH 7/1% sodium dodecyl sulfate, in which native TBSV dissociated readily into slowly sedimenting products (8). The lower ultrasonic absorption in TBSV solutions as a result of crosslinking is shown in Fig. 4.

Evaluating Characteristic Times. We cannot conclude from the ultrasonic spectra whether the relaxations are characterized by a single relaxation time or a distribution. However, a lower limit of a characteristic time $\tau$ associated with the relaxation process detected in BMV capsids can be set at $5 \times 10^{-7}$ s. Pressure-jump experiments on BMV carried out at 23°C (data not given here) have shown that the characteristic times are shorter than the time resolution of the cell, which is of the order of $5 \times 10^{-4}$ s. At 50°C, however, the relaxation frequency of the total effect in BMV was shifted into the range of observation of our ultrasonic equipment, and Fig. 5 shows that $\tau$ was of the order of $10^{-7}$ s. A more precise evaluation of the relaxation times under various experimental conditions would require use of a shock tube to explore times intermediate between those attainable with the pressure-jump and the ultrasonic techniques. It should then be possible to characterize the new relaxational contribution to the ultrasonic absorption. With TBSV, ultrasonic measurements were possible for the virus only. At 23°C, the absorption was lower than for BMV (compare Figs. 2 and 4). Unlike BMV, TBSV has a relaxation frequency that remains outside our range of observation at 50°C (see Fig. 5). Thus the corresponding $\tau$ is longer than $5 \times 10^{-7}$ s.

Occurrence of Relaxation Close to the Dimer–Capsid Transition in BMV. Fig. 1 shows that at each of the lowest frequencies (0.6 MHz and 0.9 MHz) the experimental point obtained at pH 5.6 lies above the curve. The observation of such a maximum in Fig. 1 could be a manifestation of a relaxation process that occurs close to the dimer–capsid transition. Furthermore, the mid-transition in Fig. 1 shifted towards lower pHs when the frequency $N$ was decreased. This may be another manifestation of this extra relaxation process.

Relaxation in the vicinity of the dimer–capsid transition in BMV may be due to a perturbation by the ultrasonic wave of the association–dissociation equilibrium. However, it may also be due to a postassociation structural change (reannealing) of the capsid.

CONCLUSIONS

The excess ultrasonic absorption over that of the sum of contributions of components alone, which we have observed in BMV as well as in BMV capsids, appears to be specific to the self-assembled protein shell. Very likely we have obtained evidence of spontaneous motions within an assembled system. Such motions could consist of cooperative structural changes involving either a few protein molecules or else the whole shell. The spontaneous motions (or fluctuations) we have detected may play a role in liberating the RNA and may therefore be of functional significance at an early stage of viral infection.

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