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Biophysical Chemistry 107 (2004) 101–106

Biophysical Chemistry

www.elsevier.com/locate/bpc

Can the RNA of the cowpea chlorotic mottle virus be released through a channel by means of free diffusion? A test in silico

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Received 9 April 2002; accepted 14 January 2003

Abstract

Cowpea chlorotic mottle virus (CCMV), a plant virus which is member of the *Bromoviridae* family, is used as a model for the diffusion of a random, short, single stranded RNA, [5′-R(PGpGpApCpUpUpCpGpGpUpCpC)-3′)], through a channel on the pseudo-three-fold axis using molecular dynamic simulations. This proposition is based the fact that CCMV undergoes a dynamic structural transition as a response to changes of pH, temperature and ionic strength. Results indicate that the RNA looses its secondary structure and moves into the capside channel by free diffusion. These results are congruent with the hypothesis suggesting that the CCMV capside does not have to dissolve in order to release the RNA into the host.

Keywords: Molecular dynamic; CCMV; Release RNA through channel's virus; Diffusion

1. Introduction

The cowpea chlorotic mottle virus (CCMV, bean yellow stipple virus, virus moteado amarillo) is a bromovirus that affects species from nine different families of plants, producing necrotic lessions [1] and is transmitted by some species of coleopteran insects. The CCMV has a relatively small size, bearing a capside diameter of only 26–28 nm. The 32 conspicuous capsomeres have 180 identical coat proteins, each 189 residues long [2]. Each protein subunit shares an eight-stranded β-barrel core [3] that is highly conserved among spherical viruses, and it is found in plants, bacteria, and animals as well [4–6].

Inside the capside, three unique, single stranded, positive-sense RNA molecules with 3.171 kb, 3.1 kb and 2.173 kb make all of the virus genome. When infecting the cell, the RNA molecule goes from being inside the viral capside to the host, by means of a variety of mechanisms that have been described in different models [7,8]. Perhaps the most traditional model is that in which the capside

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doi:10.1016/S0301-4622(03)00193-5

is dissolved when the cell infection starts, and the RNA molecule is freed to enter the host [7]. More recent models, however, like that of Munshi et al. [7], suggest that capside dissolution is not the only alternative to release the RNA. At least in nodaviruses, the RNA might be released by means of free diffusion, through a channel located on the pentameric axis of the molecular lattice formed by the viral capside. Results from other studies done on picornaviruses [8] agree with those of Munshi et al. [7], suggesting that the role of the pentameric axes is to create a hydrophobic patch that is used by the RNA molecule to leave the capside.

As happens with many other viruses, the mechanism used by the CCMV to release its RNA is not well understood. When the CCMV infects a cell and the RNA is being released, the viral particle undergoes a process of 'swelling', increasing its volume by approximately 10% (i.e. co-translational disassembly [9]). High levels of pH (i.e. usually higher than 6.5), temperature and low ionic strength (i.e. $i \leq 0.2$) are the conditions that trigger this process [3]. When the viral particle grows in volume, the diffusion of viral RNA is hypothesized to occur through channels appearing on the capside wall and resulting from the capside expansion. Up to 60 separate channels of 20 Å in diameter may appear, allowing free molecular exchange between the virus and its host cell [3,9,10]. The results of a recent in vitro study [11] supported this model, suggesting that the RNA release from the CCM virus capside occurs through the channel formed by the pentameric axis of the molecular grid. But, can the CCMV-RNA actually be released through these channels by means of free diffusion? The importance of this question is two-fold because it tackles the hypotheses on: (a) the little-known replication process of the CCMV; and (b) the capside-dissolution vs. channel-formation mechanisms suggested for the RNA release.

We think that the CCMV is an outstanding model to be used as a constrained environment for the entrapment and release of large molecules [10] because: (a) the dynamics and structural transitions of its capsid are related to pH and ionic strength conditions; and (b) the maximum diameter of channels (i.e. 20 Å), formed at the pseudo-three-fold axis of the $T=3$ quasi-symmetric molecular lattice of the capside's atomic structure, is large enough to allow an RNA molecule to pass through it. These properties also make of the CCMV an ideal subject to test the question addressed above.

In this paper we performed a molecular dynamics simulation to test if an RNA molecule moves into a channel located on the CCMV capside by means of free diffusion, that is, without using ATP. As far as we know, this is the first theoretical attempt to study this process.

2. Methodology

To perform the molecular dynamics simulation we obtained native atom coordinates of the CCMV capside from X-ray diffraction analysis data at a resolution of 3.2 Å, available at the Protein Data Bank web site (www.pdb.org). In order to reduce the time of the simulation while preserving the validity of the molecular interactions, we created atom coordinates of a hypothetical, random, very short and single stranded RNA sequence [5′-R(PGpGpApCpUpUpCpGpGpUpCpC)-3′] (briefly ssRNA*), using White's software (N.B. White, unpublished program). The total number of atoms in our simulated system was 3435.

All calculations were carried out using Cerius2 (version 3.5, Molecular Simulation, Inc., California, USA) running on a Silicon Graphics Origin 2000 workstation. The parameters of Amber (see http://www.amber.ucsf.edu/amber), force field simulation software that is specially designed for proteins and nucleic acids were set according to http://www.amber.ucsf.edu/amber/amber.html.

Atomic calculations for non-bonding van der Waals and electrostatic interactions were performed at a cut-off distance of 14 Å. Partial charges of the ssRNA* molecule and the CCMV channel were calculated using Amber. We did not use any explicit water molecules, but we used a distance-dependent dielectric constant to introduce in the model the effects of the solvent. Hydrogen atoms were added automatically using Cerius2. All simulations were carried out modeling all the atoms at 300 K.

Just before the simulation started, we placed the ssRNA* molecule at 12 Å from the internal side

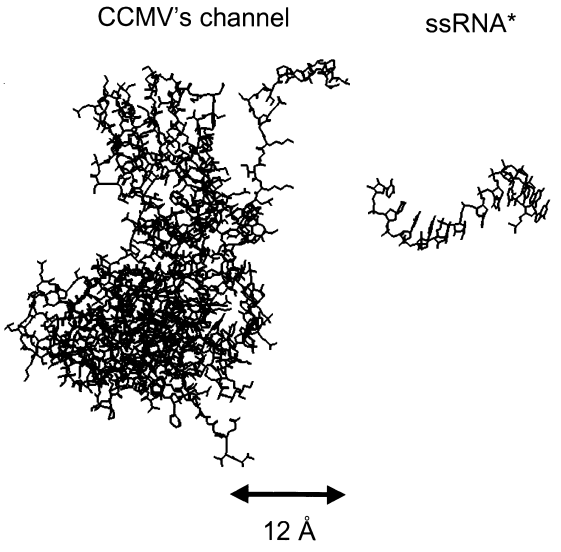

Fig. 1. Initial molecular conformations before docking. The ssRNA* molecule is at 12 Å from the virus channel.

of the capside channel (Fig. 1) to allow non-bonding interactions to initiate the diffusion process of the molecule through the capside. When the simulation began, the distance between the ssRNA* and the CCMV capside was minimized down to 50 000 steps using the conjugated gradient method, with an energy tolerance of 0.001 Kcal/mol. We allowed the channel to fluctuate between 10 and 15% of its diameter.

The molecular dynamics simulation was regarded as complete once approximately half the ssRNA* molecule had passed through the channel. This happened at 250 ps.

3. Results and discussion

The underlying principles for the viral mechanism of RNA release are mostly unknown and few data about this process are available in the technical literature. As far as we have been able to find, this is the first attempt to simulate the diffusion driven release of an RNA molecule through the CCMV capside.

Fluctuations of the total energy of the ssRNA* molecule along the simulation can be divided into three different regions in which the energy is constant (Fig. 2). Given that the energy is constant at points in which the ssRNA* molecule looses its secondary structure, these regions indicate the different conformations of the ssRNA* while passing through the capside channel (Fig. 3a,b, and c). The reduction of energy is congruent with that expected in a non-ATP-mediated process of diffusion. The final conformation of the ssRNA* going through the virus channel is shown in Fig. 3c.

The total energy fluctuations are congruent with the dynamics of the radius of gyration of the molecule (Fig. 4). After an initial 1.0% increase during the first 100 ps of the simulation, the radius of gyration immediately changed from 15.0 to 11.5 Å. After 150 ps the radius remained stable and averaged 11.2 ± 0.2 Å after the last 200 ps of the simulation.

The conformation of the ssRNA*–CCM virus complex (supplement material Fig. 5), the molecular dynamics and the energy values obtained by this simulation indicate that the ssRNA* can actually move into the simulated capside channel by means of free diffusion, and part of the molecule can pass through it. These results are congruent with the hypothesis suggesting that the CCMV

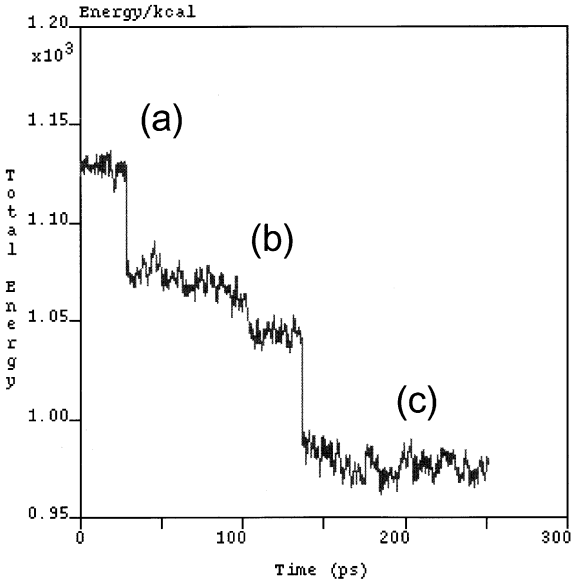

Fig. 2. Total energy vs. time (up to 250 ps) of the ssRNA* diffusing through the channel's virus.

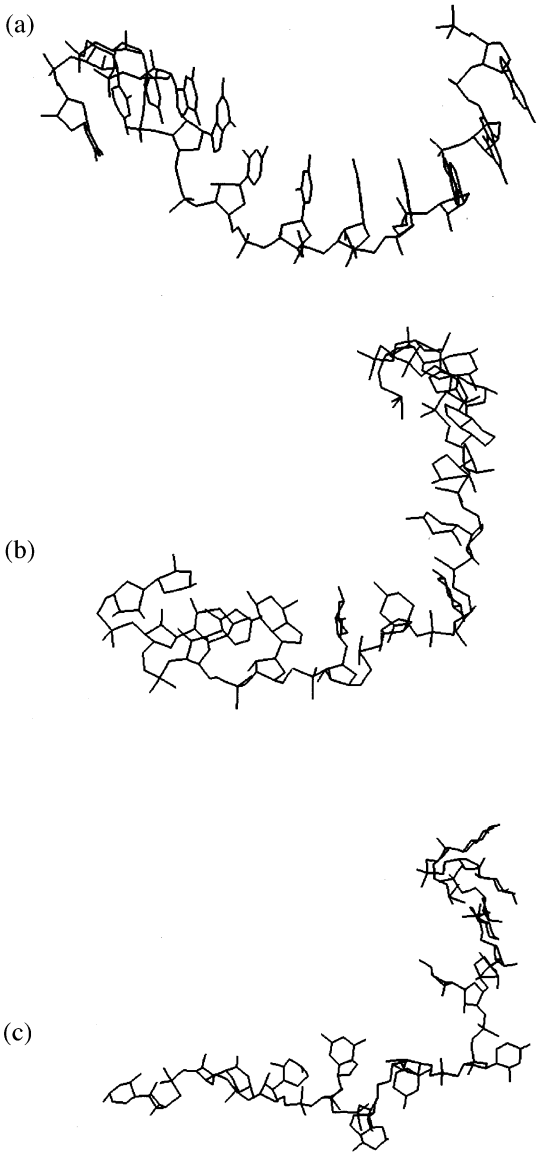

Fig. 3. Initial (1 ps, a), mid (110 ps, b) and final (250 ps, c) structures of ssRNA* according to our molecular dynamics simulation.

capside does not have to dissolve in order to release the RNA into the host.

Even if this theoretical approach offers a glimpse to the release of RNA through the CCMV capside, it is possible that some of these results are a consequence of the conditions set for the simulation. We think that forces related to molecular diffusion are probably not sufficient to explain the loss of the secondary structure of the ssRNA*. The secondary structure of the RNA depends on the non-bonding interactions and they become very weak when water molecules are absent. Then, it is possible that the ssRNA* will not completely loose its secondary structure during diffusion in a system in which water molecules are simulated. The size of the ssRNA* molecule raises another important issue about these results. Our ssRNA* molecule is only 10% as long as the real RNA molecule. Under a more realistic scenario, is possible that an energy-mediated process might be involved releasing a larger and fully structured RNA. There, an ATP-mediated diffusion should be considered as a testable alternative in the simulated system. For these same reasons, and because simulations were carried out until only half the ssRNA* molecule passed through the channel, we will not speculate about the dynamics and the structure of the ssRNA* molecule once it is on the other side of the capside.

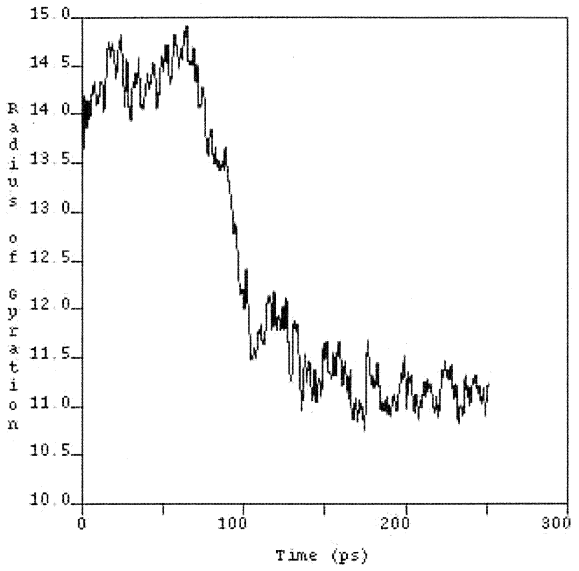

Fig. 4. Radius of gyration of RNA vs. time of coordinates of Cα atoms during the molecular dynamics simulation (up to 250 ps).

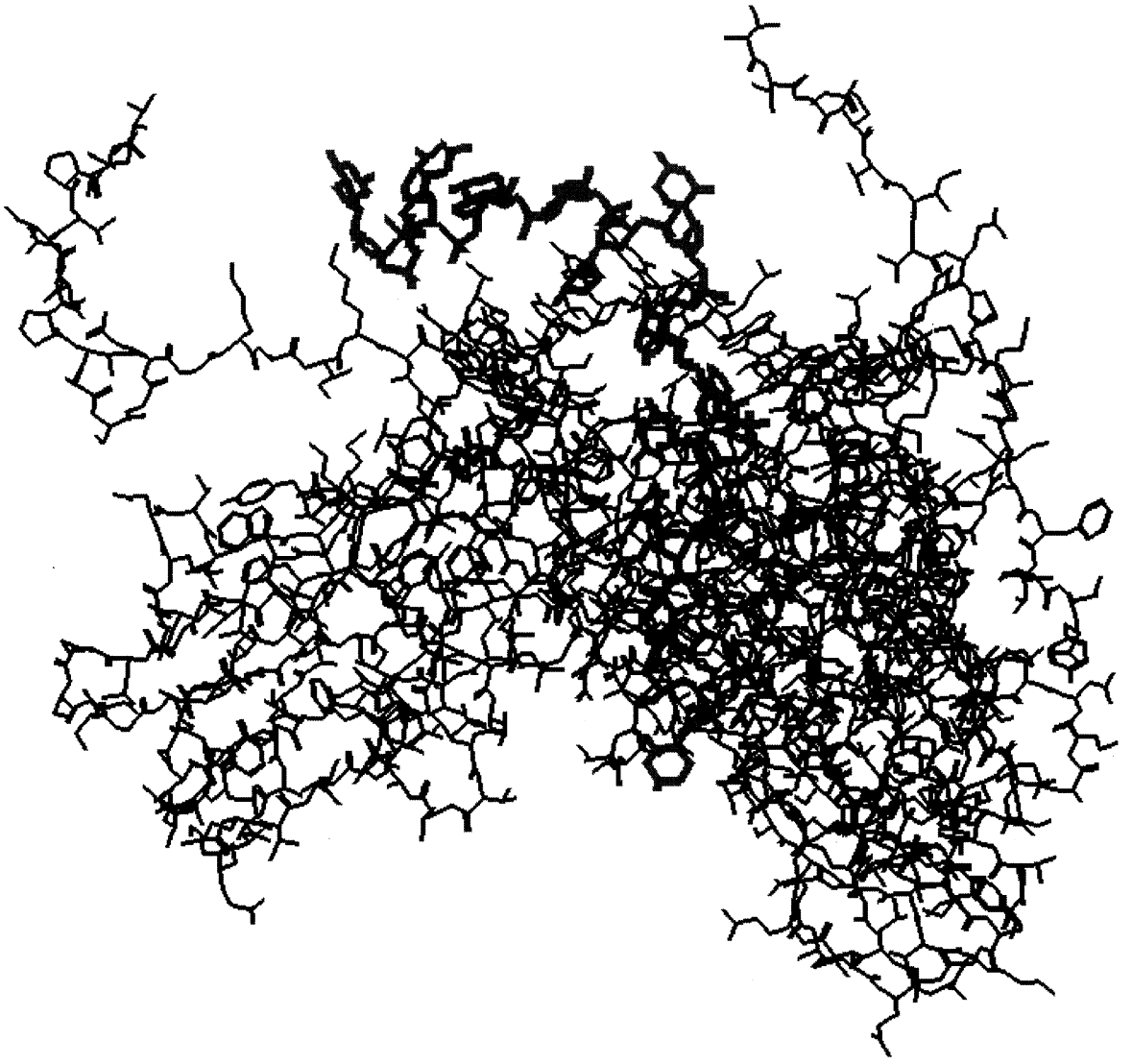

Fig. 5. (Supplement material). Snapshot of the final conformation of ssRNA*-CCMV complex in the last step of the molecular dynamics simulation (after 250 ps). The blue color indicates the viral channel and the red color indicates the ssRNA* molecule.

Even though many of the mechanisms represented in this study remain to be improved and tested in future simulations, we think that this first approach demonstrates that a simple molecular dynamics process can be partially and potentially responsible for initiating the release of the RNA molecule from the CCMV capside.

Acknowledgments

R.I. gratefully acknowledges Dr Hong Zhou's comments on an earlier draft of the manuscript. The authors acknowledge CeCalCULA (Centro Nacional de Cálculo Científico Universidad de Los Andes) for providing all the computational sup-

port. This project was partially funded by CAVEFACE (Cámara Venezolana de Fabricantes de Cerveza).

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